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THE AMERICAN JOURNAL OF PATHOLOGY

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AURICULAR ENDOCARDITIS OF RHEUMATIC ORIGIN *

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In recent publications, attention has been directed by MacCallum[1] to certain changes occurring at times in the wall of the left auricle in rheumatic disease of the heart. The appearance of the lesion as described by him is very characteristic. Upon the wall of the auricle above the root of the posterior leaflet of the mitral valve, the endocardium is thickened, forming a corrugated patch, often with a rough, dull, fibrinous layer on the surface; at other times the surface seems not to be roughened by any thrombotic mass. In some instances the thickened patches appear to be very dense and scar-like as though representing a late or healing stage of an earlier process. Upon section, the great thickening of the lining wall of the auricle inside the muscular layer is readily seen. Histologically, the changes are equally characteristic. In the tissue about the auriculoventricular junction, and in the wall of the auricle, as well as in the roots of the valve, there is great edema and marked infiltration with wandering cells of many sorts. Most of these are mononuclears, but there are also many polymorphonuclear leucocytes, as well as numerous large Aschoff bodies composed of the typical cells. Because of the arrangement of the elastic lamellae, the Aschoff cells are forced into rows, giving the bodies a banded appearance. The Aschoff bodies are numerous throughout the whole extent of the patch of thickening, even extending into the sinus tissue and into the roots of the valve. The innermost layer of the auricular wall frequently has a

* Received for publication January 5, 1926.

hyaline appearance over the involved area; upon its surface is a dense film of fibrin. Outside the elastic layer the tissue reaching to the myocardium is densely infiltrated with wandering cells of various types, including in some cases a number of eosinophiles.

In 1898, Claude and Levaditi [2] demonstrated a case of chronic mitral endocarditis with what are described as "ulcerations" on the wall of the left auricle, the ulcerations being most abundant near the mitral orifice and reaching to the openings of the pulmonary veins. There were no thrombi on them; the surface was yellowish or gray in color, and calcium was deposited in the superficial portions. Histologically, capillaries were found to extend almost to the base of the ulcerations; and many round cells surrounded the capillaries. At other points the round cells were present together with elongate or stellate connective tissue cells. "Granular cells of Ehrlich" were seen also. Some of the smaller arteries showed marked endarteritis with almost complete obliteration of the lumen, and at the periphery of some of the vessels was a proliferation of granulation tissue. In the myocardium, granulation tissue was most marked about the large capillaries. The pericardium contained numerous vessels presenting the same inflammatory alterations as in the endocardium and myocardium. Bacteria could not be demonstrated. It is unfortunate that a history is not included with the report. In view of the fact that bacteria could not be demonstrated, that the mitral valve showed only chronic changes without a recent endocarditis and that there were no thrombi on the "ulcerations," it seems very probable that this case is one of rheumatic endocarditis of the auricle with calcification. Furthermore, the nature of the lesions, though incompletely described, in the myocardium and pericardium would tend to support this belief.

Harper [3] in an address before the Southern Medical Association at Lexington, Kentucky (1912), described the heart of a boy, 8 years of age, which he had studied in the Great Ormond Street Hospital for Sick Children, London. The child had had chorea. There were verrucous vegetations on the mitral and aortic valves, and the "endocardium of the left auricle was found to be thickened and wavy. This was considered evidence of a former endocarditis." (Case VII.) The report does not include a histologic examination. This was very probably a case of rheumatic endocarditis of the auricle, but the evidence presented is too scant to allow one to be certain.

In 1920 Hertel[4] in a discussion of parietal endocarditis gives a detailed description of a case of verrucose endocarditis with involvement of the left auricular wall (Case VI). Her description presents clearly the gross picture and histologic changes. Upon the mitral valve were vegetations, in part wart-like and in part more polypoid. These vegetations extended to the base of the valve, and from there were continued upon the auricle. The surface of the auricle in places was smooth; in other places there were parallel, transverse or irregularly running ridges and upon and between them, warty masses. The verrucae and ridges extended high up into the auricle. The foramen ovale could scarcely be recognized.

Histologically, the verrucae were made up of fibrin. In the deeper part of the auricular endocardium were bands of connective tissue, staining red with Van Gieson's picro-fuchsin stain, while near the surface the connective tissue stained less intensely with the fuchsin, even appearing in places yellow. Small masses of cells were present, made up of connective tissue cells, polymorphonuclear leucocytes, lymphocytes and plasma cells; such cells being found in the superficial as well as the deeper parts of the endocardium. Many capillaries invaded the endocardium from the myocardium in a perpendicular fashion, and were surrounded by mantles of these cells. Bacteria could not be found. The continuity between the lesions of the mitral valve and those of the auricular wall is emphasized in the discussion of the case. The clinical history is not included in the report, but the case is undoubtedly one of rheumatic disease with involvement of the wall of the left auricle. The lesion as described would seem to coincide with the later stages in rheumatic auricular endocarditis.

Swift[5] states that "in the mural endocardium lesions are found without a deposit of fibrin on the surface, but showing a type of infiltration closely resembling that of Aschoff bodies." No mention is made of the situation of these lesions, nor of their frequence in the auricular and ventricular endocardium.

Since the publication by MacCallum, Stewart and Branch[6] have described a case of rheumatic endocarditis of the left auricle, with calcification and Aschoff bodies.

In the past five years there have been thirty-one cases of rheumatic valvular endocarditis demonstrated at necropsy in this hospital, and of these, nine have shown involvement of the auricular endocardium.

Gross Pathology of Rheumatic Auricular Endocarditis

The gross appearance of the auricular rheumatic endocarditis is as distinctive as that of the verrucose vegetations on the valves, or the myocardial and pericardial changes.

In the simplest form the lesion is confined to an area just above the non-aortic or posterior leaflet of the mitral valve. Here the endocardium is made irregular by low ridges or hillocks often closely crowded together to form a plaque 3 cm. or more in extent. At other times these elevations are more widely separated from each other. When closely aggregated, the surface has a furrowed appearance and the furrows, while having no definite pattern, frequently run roughly perpendicular to the ring of the valve (Figs. 1 and 2). Similar, but somewhat less distinct, ridges can usually be traced downward across the auricular surface of the valve leaflet, to merge with the vegetations near the margin of the cusp (Fig. 1).

The surface of these auricular elevations may be quite smooth and glistening, or slightly dull and irregular; it is occasionally roughened by tiny, dull, yellowish flecks or a little blood clings to it. Rarely, more distinct projections resembling vegetations are seen. The color is variable also. In the more acute lesions these thickenings are tawny gray; in the older stages, grayer and translucent.

When the process is more extensive, flat or delicately ridged plaques of yellow color are found in many parts of the auricle. They often surround the orifices of the pulmonary veins but do not extend into them. Seldom is the surface of the fossa ovalis involved. In one instance almost the entire endocardial surface of the auricle was lifted up to form a single, flat plaque with a faintly wrinkled surface (Fig. 3). This condition apparently resulted from the coalescence of many smaller areas. Not infrequently the elevations are to be seen in the first portion of the auricular appendage. The edges of these elevated areas may be quite abrupt or they may fall away in an almost imperceptible fashion.

When a section is made through the areas of thickening, certain changes can be readily discerned. The endocardial layer is found to be considerably increased in thickness; it is gray or yellowish gray, the yellow color being most pronounced in the more acute lesions. In some instances, only the very superficial portion seems to be the site of the changes, for the gray, translucent, normal-appearing

endocardium is easily traced as a regular layer beneath the superficially placed yellowish mass. Yet, from the histologic study of such cases, it is found that this normal-appearing portion of the endocardium beneath the plaque is often much altered. In other instances the endocardium is involved throughout its entire width, for it no longer appears as an even gray band but is irregular and distorted. Little irregularity can be made out, however, in the outline of the lower or myocardial border.

In the later stages these hillocks and ridges flatten down and frequently become less distinct (Figs. 4 and 5). They are readily recognized as pale gray thickenings, sometimes containing thin plates of calcium. Section through these raised areas shows that the endocardium is widened, dense, homogeneous and gray, with the deposits of calcium in the superficial part.

Histology of the Normal Endocardium

The endocardium of the left auricle has been described by Königer [7] and separated by him into the following layers: (1) endothelium, (2) a delicate subendothelial layer of connective tissue, (3) a thick layer of elastic fibers, with connective tissue, and (4) a connective tissue layer with abundant elastic fibers.

Nagayo [8] further extended the differentiation of the auricular endocardial structure, and divided the endocardium as follows: (1) endothelium, (2) inner connective tissue layer, (3) middle layer of elastic and connective tissues, and (4) outer connective tissue layer, with a few coarse elastic fibers. Between the last two layers he demonstrated the presence of smooth muscle, either as scattered fibers or more or less collected together into bundles. He aptly likened the structure of the endocardium to that of the entire wall of the larger arteries.

It has seemed to us that the innermost elastic fibers are often collected into a fairly definite bundle resembling the internal elastic lamella of the larger arteries.

The structure of the endocardium of the right auricle is similar to that of the left, though in the former situation the endocardium is nowhere as thick as in the latter.

Histology of Rheumatic Auricular Endocarditis

At the site of the thickened areas, great changes are to be made out in the endocardium. In many of the more acute lesions, large accumulations of small mononuclear cells, many polymorphonuclear leucocytes and occasionally a few eosinophiles are found in the outer half of the endocardium. At times these infiltrations reach to the inner surface.

More striking than these are collections of large cells generally forming a palisade along a band of hyaline material, their nuclei being perpendicular to the band (Figs. 6, 7 and 8). Frequently this band of hyaline substance coincides with the layer of connective tissue just beneath the endothelium. In some instances this subendothelial connective tissue is swollen and hyaline over a considerable area without any cells being accumulated about it. At other times similar though shorter hyaline bands lie in the elastic-connective tissue layer. Occasionally one of these bands will bend abruptly, almost at right angles, yet these larger cells follow its turnings and preserve their perpendicular position with regard to it.

In addition to the striking palisade arrangement of these cells, smaller groups of them are found which have a radial arrangement about a central mass of the hyaline material, probably the result of a transverse instead of a longitudinal section of the band about which they are accumulated. Somewhat similar, though less regularly arranged, accumulations of these large cellular elements are collected about foci consisting of fragments of swollen collagen.

The cytoplasm of these prominent cells stains faintly blue in the hematoxylin-eosin preparations. The nuclei are vesicular and oval, or occasionally slightly lobed, and some contain a large stellate nucleolus; in others the chromatin is in smaller particles. The nuclear membrane is wide and stains deeply. In preparations stained with methyl green-pyronin, many of these cells stand out sharply because of the brilliant red cytoplasm and pale green nucleus. They resemble in morphology and staining reaction the large cells taking part in the formation of the Aschoff bodies in the myocardium and other places. These lesions probably are modified Aschoff bodies, their shape being determined by the laminated structure of the tissue in which they occur. In sections treated with Weigert's elastic tissue and hematoxylin-Van Gieson's picro-fuchsin stains, the

material about which these cells are accumulated stains intensely with fuchsin and is obviously swollen, degenerated collagen. Accompanying this degenerated collagen are occasionally well preserved or slightly swollen elastic fibers, the cells then surrounding both.

Other sections at times show only cellular accumulations of another sort. These are made up of cells with indistinct outline and having compressed, distorted, curved and elongate nuclei. With them are many polymorphonuclear leucocytes, a few plasma and small round cells, occasional eosinophiles and also pale cells with vesicular nuclei containing a few small particles of chromatin (Figs. 9 and 10). The number of polymorphonuclear leucocytes is quite variable; in some instances they are so numerous that they are the predominant cells, tending to obscure the other cellular elements.

These cell aggregations may be found in any portion of the endocardium, but are perhaps more often present in the inner half, or even between the main elastic-connective tissue layer and the endothelium. The distorted cells are most probably the result of degeneration of the large pale cells with vesicular nuclei, which are apparently large mononuclear wandering cells. They do not correspond morphologically with the Aschoff cells, neither do they stain with methyl green-pyronin as do the Aschoff cells. Furthermore, as a rule, no Aschoff cells can be demonstrated with the methyl green-pyronin stain in these cell masses.

The arrangement of the large distorted cells in such accumulations is characteristic. Their nuclei are usually directed perpendicularly to the endocardial surface. Cell degeneration takes place readily, for nuclear fragments lie scattered about. These cell aggregations are often the cause of the ridges and the irregularities of the auricular surface.

The endothelium over the sites of these changes is frequently intact. At other times there is a thin layer of fibrin upon the surface where the endothelial cells have disappeared, and now and then this fibrin is collected into verruca-like vegetations (Fig. 11).

The elastic fibers are widely separated and pushed apart by the various cell accumulations. Often the fibers are slightly swollen and fragmented, or they may pass intact through the cell mass, though somewhat bent out of their usual course. The internal elastic lamella can be readily made out either passing through a mass of the infiltrating cells or curving upward over them and preserving

its normal relationship to the endothelium (Fig. 12). Sometimes the fibers of this lamella are spread apart by the cells, the innermost fibers passing over the accumulated cells, the remainder through the cell mass, uniting on either side of it. Now and again the innermost fibers are ruptured. Delicate elastic fibrils, possibly newly formed, can be discerned between the cells.

In the acute stages there is considerable edema, and fibrin is found in varying amounts among the distorted cells, but none can be demonstrated in the homogeneous material about which the Aschoff cells are gathered. Fibrin is readily demonstrated in places upon the surface of the endocardium over those areas where the endothelium has disappeared, and is also present in considerable quantity in the edematous vascular zone near the myocardium.

Bacteria have not been demonstrated in either Gram or Levaditi preparations.

Healing and repair take place readily. From the myocardium, capillaries penetrate into the outer half of the endocardium but they have not been found to pass beyond the mid-portion of it. Surrounding these new vessels are many small mononuclear cells, plasma cells and a few polymorphonuclear leucocytes. In the superficial part, especially between the main elastic-connective tissue layer and the endothelium there appear cells resembling fibroblasts with their nuclei directed toward the surface (Fig. 13). These cells form an avascular tissue, frequently staining yellow with the Van Gieson picro-fuchsin stain. The fibrin upon the surface of the endocardium is invaded and surrounded by such cells, hyaline masses of it being seen in the superficial part of this new tissue. In this compact new tissue, delicate elastic fibrils appear, often turning upward like a fringe into the deepest portion (Fig. 14). The internal elastic lamella may pass over such a scar, giving the impression that the scar marks the site of one of the accumulations of distorted cells and polymorphonuclear leucocytes; usually it passes beneath the plaque. Calcium is frequently deposited in the superficial portion of the endocardium where the scarring is taking place, and it may be in thin plates or small nodular clumps.

The elastic fibers in the mid-portion of the endocardium are stretched and taut beneath the new tissue, and recall very strikingly the appearance of the elastic fibers beneath a sclerotic plaque in the aorta.

As the healing progresses, the characteristic cells disappear and finally there is left the dense avascular scar in the superficial part, sometimes containing calcium. Also occasional collections of small wandering cells are found at the junction of the endocardium and myocardium; such cell aggregations appear to persist for a long time. The end result gives little indication of the striking cell infiltration and accumulations which have gone before.

It is not to be supposed that the changes enumerated above take place in definite stages and are carried through to completion before other acute lesions appear. Acute, healing and healed lesions are frequently encountered in the same section indicating that there have been repeated insults. Neither does one usually find the various cell types occurring to the exclusion of the others. MacCallum has emphasized the presence of the Aschoff cells and bodies as being the most characteristic feature, and they do form a conspicuous part of the lesion. In the case reported by Stewart and Branch,[6] only Aschoff bodies and calcium deposits were found. From our studies it has seemed to us that the masses of distorted cells and polymorphonuclear leucocytes constitute a feature as prominent and distinctive as the Aschoff bodies. It is probable that many of the plaques of calcium found in the auricular endocardium in chronic mitral disease mark the sites of previous acute rheumatic lesions.

Rheumatic Endocarditis of the Right Auricle

Only one instance of rheumatic endocarditis of the right auricle has come to our attention. In this case with extensive endocardial changes in the left auricle, a chain of tiny verrucae was present along the anterior margin of the fossa ovalis.

From the foregoing study, one is convinced that there occur in the endocardium of the auricle, lesions which are distinctive, both grossly and histologically, of rheumatic endocarditis. The endocardium of the left auricle is more frequently the site of the lesions than that of the right auricle. In the series of cases studied, the left auricle was involved in approximately one-third of the cases of rheumatic valvular endocarditis.

I am indebted to Dr. Walter W. Palmer for permission to transcribe the clinical records, and to Mr. Alfred Feinberg for the drawings.

Résumé of Cases

Case I. Acute Rheumatic Endocarditis of Left Auricle.

Autopsy 9168, history 51744. L. D., male, age 14. One attack of rheumatism, duration two weeks, seven years before death. Scarlatina four years ago. Dyspnea on exertion for two years.

Heart: Greatly enlarged. Pericardium everywhere adherent to heart. Verrucose vegetations on tricuspid, mitral and aortic valves. Mitral valve scarred. Endocardium of left auricle thickened to form a large plaque whose surface is somewhat wrinkled and irregular. The elevated area is yellow in color. The thickening involves almost the entire auricle, extending into the auricular appendage and downward upon the surface of the mitral valve (Fig. 3). The process stops at a little distance from the orifices of the pulmonary veins. Myocardium scarred.

Histologic Examination: In the endocardium of the left auricle are bands of hyaline material bordered by Aschoff cells. There are also Aschoff cells about collagen fragments. Large areas densely infiltrated with small mononuclear cells, polymorphonuclear leucocytes and a few eosinophiles; capillaries are penetrating from myocardium into these cell accumulations. No vegetations on the surface. Elastic fibers are pushed apart by the infiltrating cells, and are distorted and fragmented. Delicate elastic fibrils in the areas of cell infiltration. Aschoff bodies in the myocardium.

Case II. Healed Rheumatic Endocarditis of Left Auricle.

Autopsy 9179, history 45652. L. W., male, age 6. Acute rheumatic fever at 4 and 5 years of age. Mitral insufficiency.

Heart: Enlarged. Fibrinous pericarditis. Tricuspid valve thickened. Endocardium above posterior leaflet of mitral valve is thrown up into low gray ridges, with smooth and shining surface. Mitral and aortic valves scarred.

Histologic Examination: Focal accumulations of small round cells at junction of endocardium and myocardium. In mid-portion of endocardium small groups of cells, with indefinite outline, large vesicular nuclei and stellate nucleoli. Internal to the elastic-connective tissue layer is a mass of loose avascular connective tissue, within which are a few delicate elastic fibers. Aschoff bodies in myocardium. Organizing fibrinous pericarditis.

Case III. Healed Rheumatic Auricular Endocarditis.

Autopsy 9370, history 32823. J. V., male, age 6. Measles and chickenpox. Tonsillitis repeatedly. Attacks of rheumatism three, two and one years before death.

Heart: Enlarged. No pericarditis. Verrucae on mitral and aortic valves. Above posterior leaflet of mitral valve the endocardium of the auricle is thickened to form rounded low hillocks with gradually sloping margins and smooth surface (Fig. 4). No scars visible in the myocardium.

Histologic Examination: Between the internal elastica and the endothelium are accumulations of avascular fibrous tissue, with nuclei directed perpendicularly to the endothelial surface. Elastic fibers do not appear irregular. Aschoff bodies in myocardium.

CASE IV. ACUTE RHEUMATIC ENDOCARDITIS OF LEFT AURICLE.

Autopsy 9408, history 56872. M. F., female, age 3. Acute rheumatic fever, knees and ankles involved, one year before death. Scarlatina shortly after the rheumatic fever. Dyspnea on exertion for four months.

Heart: Hypertrophied. Pericardium everywhere adherent to heart. Verrucae on tricuspid, mitral and aortic valves. Endocardium of left auricle above posterior leaflet of mitral valve is thickened over an area of 3 cm., the plaque reaching into first part of appendage and across valve leaflet to line of closure (Fig. 1). Surface of this thickened area traversed by many shallow depressions. Plaque is pale yellow; surface is only slightly less smooth than that of the endocardium in uninvolved portion of the auricle. Many smaller tawny yellow plaques in other parts of the auricle, near fossa ovalis and orifices of pulmonary veins. Sections through these plaques show the deeper portion of the endocardium to be slightly opaque, while the bulging plaque is edematous and yellowish white in color. Myocardium scarred.

Histologic Examination: The changes are quite identical with those described in Case I. Numerous Aschoff bodies and large scars in the myocardium.

CASE V. HEALED AND ACUTE RHEUMATIC ENDOCARDITIS OF LEFT AURICLE.

Autopsy 9471, history 58720. W. G., male, age 27. Acute rheumatic fever at age of 21, with cardiac involvement. Palpitation for two months before first admission to hospital. One year later dyspnea and orthopnea.

Heart: Greatly enlarged, weighing 1080 gm. Pericardium everywhere adherent to heart. Mitral valve thickened. Verrucae on mitral and aortic valves. Above the posterior leaflet of the mitral valve, over an area 4×2 cm., are many elevated yellowish plaques and ridges. Similar though smaller yellow ridges extend downward from this large aggregation across the auricular surface of the posterior leaflet of the valve (Fig. 2). The margins of the plaques are quite abrupt at the upper border. Surface of the plaques is slightly dull in a few places. Section shows that the thin gray endocardial layer widens out at the sites of these plaques, yet it can be traced as a gray band beneath the superficial yellow portion. Along the anterior margin of the fossa ovalis in the right auricle is a row of tiny verrucae.

Histologic Examination: Characteristic rheumatic valvular endocarditis. In some places on the surface of the auricle are small hyaline masses which are being surrounded by a loose connective tissue. In other places between the elastic-connective tissue layer and the endothelium are masses of connective tissue cells with their nuclei directed perpendicularly to the surface (Fig. 13). Delicate elastic fibrils pass up into this connective tissue. No Aschoff bodies found in several sections of the myocardium.

CASE VI. ACUTE RHEUMATIC ENDOCARDITIS OF LEFT AURICLE.

Autopsy 9529, history 60354. A. S., male, age 14. No history of rheumatism. Frequent attacks of sore throat. Precordial pain, dyspnea and fever one year ago.

Heart: Pericardial sac everywhere bound to the heart by fibrous adhesions. Heart enlarged. Verrucae on the tricuspid, mitral and aortic valves. Delicate elevated yellowish plaque extends across the auricular surface of the posterior leaflet of the mitral valve and merges with similar larger plaque on the endocardial surface of the auricle. This latter plaque appears to be formed by the

coalescence of many smaller ones. The surface is distinctly roughened and dull, and is flecked with red. The plaque reaches to the openings of the pulmonary veins. Isolated oval or rounded elevated areas, 3 to 4 mm. in diameter, occur in other parts of the auricle. One of these at the orifice of the auricular appendage is somewhat larger and is continued downward across the auricular surface and upon the anterior leaflet of the mitral valve. On section the main portion of the endocardium passes in a regular fashion beneath the gray translucent thickenings in the superficial part.

Histologic Examination: The lesion is intense. Aschoff cells are collected about bands of hyaline material (Fig. 6). In other areas there is intense infiltration with polymorphonuclear leucocytes, mononuclear cells, a few eosinophiles and aggregations of cells with distorted compressed elongate nuclei (Figs. 9 and 10). The nuclei of these latter cells are directed perpendicularly to the surface. The endothelium is intact over some of these cell accumulations; in other places masses of fibrin are on the surface (Fig. 11). In yet other places are masses of avascular connective tissue between the endothelium and main elastic-connective tissue layer, with many delicate elastic tissue fibrils in the deeper part of the connective tissue mass (Fig. 14). The elastic fibers are spread apart and fragmented where these various cells are accumulated (Fig. 12). Delicate elastic fibrils are found in the cell masses. The internal elastica is lifted up by groups of the distorted cells. Many capillaries arising in the myocardium are invading the outer portion of the endocardium and are surrounded by small round cells, plasma cells and a few polymorphonuclear leucocytes and eosinophiles. The myocardium just beneath the endocardium is edematous. Many Aschoff bodies are found in the myocardium.

CASE VII. HEALED RHEUMATIC ENDOCARDITIS OF THE LEFT AURICLE.

Autopsy 9534, history 43778. K. F., female, age 37. Rheumatic fever at age of 13. Dyspnea and palpitation began four years before death.

Heart: Moderately enlarged, weighing 450 gm. Mitral valve thickened and the edge rolled under. Over the non-aortic leaflet of the valve the auricular endocardium is thrown up into irregular ridges and folds, and these are continued for a short distance across the auricular surface of the valve (Fig. 5). These thickenings are translucent and faintly yellow.

Histologic Examination: The elevations are composed of avascular connective tissue lying internal to the main elastic-connective tissue layer of the endocardium. The internal elastic lamella lies beneath these areas and is somewhat interrupted as it passes below them. Many delicate elastic fibrils are present in the connective tissue masses. Aschoff bodies occur in the myocardium.

CASE VIII. HEALED RHEUMATIC ENDOCARDITIS OF THE LEFT AURICLE WITH CALCIUM DEPOSITS.

Autopsy 9607, history 52993. J. F., male, age 17. Rheumatic fever four years before death. Dyspnea, edema of legs and palpitation for three months.

Heart: Enlarged, weight 710 gm. Tricuspid and mitral valves are thickened and have verrucae upon them. Above the posterior leaflet of the mitral valve, the endocardium of the left auricle over a large area is thrown up into irregular grayish yellow folds and rounded plaques; calcium is deposited in the superficial portion of these folds.

Histologic Examination: Internal to the main elastic-connective tissue layer, the endocardium is irregularly thickened by masses of dense connective tissue

in which many delicate elastic fibrils are found, usually directed at right angles to the surface. Here and there are coarse elastic fibers just beneath the endothelium and parallel to it; these are apparently remnants of portions of the internal elastic lamella. Calcium is deposited in the masses of dense connective tissue. Localized collections of lymphocytes are present in the endocardium near the myocardial surface.

Case IX. Acute Rheumatic Endocarditis of Left Auricle.

Autopsy 9627, history 48682. S. G., male, age 9. Two attacks of rheumatic fever at 5 and 7 years of age. Mitral and aortic insufficiency; mitral stenosis.

Heart: Greatly enlarged. Pericardial surface covered with a thick fibrinous exudate. Tricuspid and pulmonic valves normal. Left auricle is enlarged. Numerous slightly elevated yellow areas in the endocardium in many parts of the auricle, but especially marked above the posterior leaflet of the mitral valve. These yellow areas are glistening, though frequently the surface is a little wrinkled. Marked thickening and distortion of the mitral leaflets with festoons of verrucose vegetations near the free border. Aortic leaflets thickened; chains of verrucae upon them.

Histologic Examination: At the site of the plaques in the left auricle the endocardium is greatly thickened and infiltrated with cells consisting of large mononuclears, small round cells and many polymorphonuclear leucocytes. Close to the surface is a band of swollen and fragmented collagen. In many parts there are no cells about this band. In other places hyaline bands are accompanied by palisades of Aschoff cells (Figs. 7 and 8). In yet other places are dense accumulations of cells; some of the cells are large with pale vesicular nuclei, others have distorted compressed nuclei. With these are many polymorphonuclear leucocytes and a few eosinophiles. In the deeper part of the endocardium are numerous capillaries about which are large mononuclear cells, lymphocytes and a moderate number of polymorphonuclear leucocytes. The endothelium is intact on the surface. In the myocardium are great numbers of characteristic Aschoff bodies. The epicardium is covered with a thick layer of fibrin undergoing organization.

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DESCRIPTION OF PLATES

PLATES 1–9

(For more detailed description of gross specimens, see résumé of cases.)

FIG. 1. Autopsy 9408. Acute rheumatic endocarditis of mitral valve and left auricle. Adherent pericardium.

FIG. 2. Autopsy 9471. Acute rheumatic endocarditis of mitral valve. Acute and healed rheumatic endocarditis of left auricle. Adherent pericardium.

FIG. 3. Autopsy 9168. Rheumatic endocarditis of mitral valve. Extensive acute rheumatic endocarditis of left auricle. Adherent pericardium.

FIG. 4. Autopsy 9370. Rheumatic endocarditis of mitral valve. Healed rheumatic endocarditis of left auricle.

FIG. 5. Autopsy 9534. Healed rheumatic endocarditis, with great scarring and thickening of mitral valve. Healed rheumatic endocarditis of left auricle.

FIG. 6. Autopsy 9529. Acute rheumatic endocarditis of left auricle. Hyaline bands surrounded by Aschoff cells. (Hematoxylin-eosin stain.)

FIG. 7. Autopsy 9627. Acute rheumatic endocarditis of left auricle. Aschoff cells along bands of hyaline material in mid-portion of endocardium. Accumulation of wandering cells in deeper part of endocardium. (Hematoxylin-eosin stain.)

FIG. 8. High power of outlined field in Fig. 7, showing character and arrangement of cells around hyaline material. (Hematoxylin-eosin stain.)

FIG. 9. Autopsy 9529. Acute rheumatic endocarditis of left auricle. Dense infiltration of endocardium with wandering cells of many varieties. (Hematoxylin-eosin stain.)

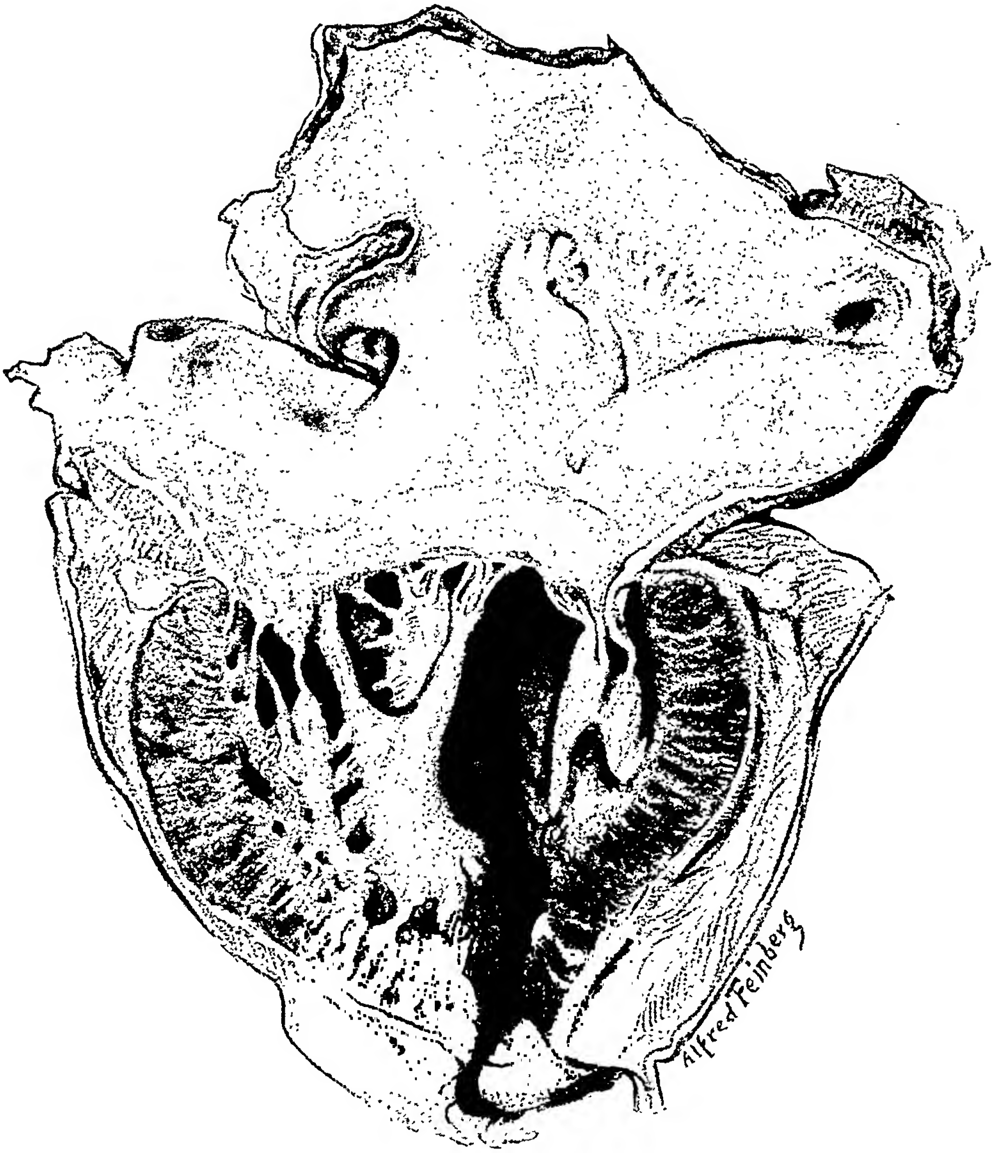
FIG. 10. High power of outlined field in Fig. 9, showing large mononuclear wandering cells, polymorphonuclear leucocytes, small round cells and cells with distorted, compressed, elongate nuclei. (Hematoxylin-eosin stain.)

FIG. 11. Autopsy 9529. Acute rheumatic endocarditis of left auricle. Vegetation composed of fibrin strands. (Hematoxylin-eosin stain.)

FIG. 12. Autopsy 9529. Acute rheumatic endocarditis of left auricle. Separation and fragmentation of elastic fibers following cellular infiltration of endocardium. (Weigert's elastic tissue-hematoxylin-Van Gieson stain.)

FIG. 13. Autopsy 9471. Healed rheumatic endocarditis of left auricle. Mass of avascular connective tissue internal to elastic-connective tissue layer of endocardium. (Weigert's elastic tissue-hematoxylin-Van Gieson stain.)

FIG. 14. Autopsy 9529. Healed rheumatic endocarditis of left auricle. Avascular connective tissue internal to elastic-connective tissue layer of endocardium. Fringe of delicate elastic fibrils in deepest part of scar. Elastic fibers underlying new tissue stretched and taut. (Weigert's elastic tissue-hematoxylin-Van Gieson stain.)



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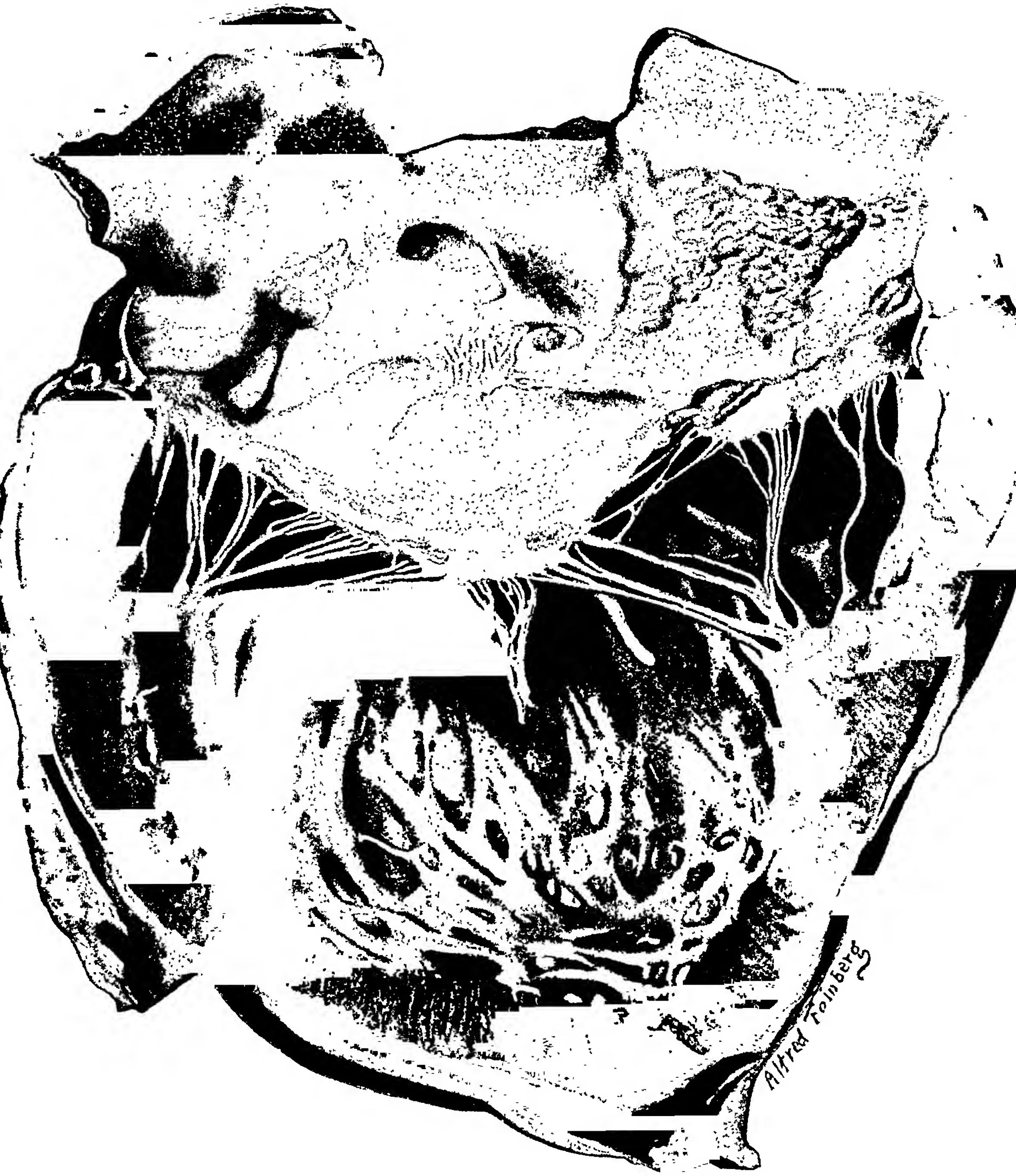

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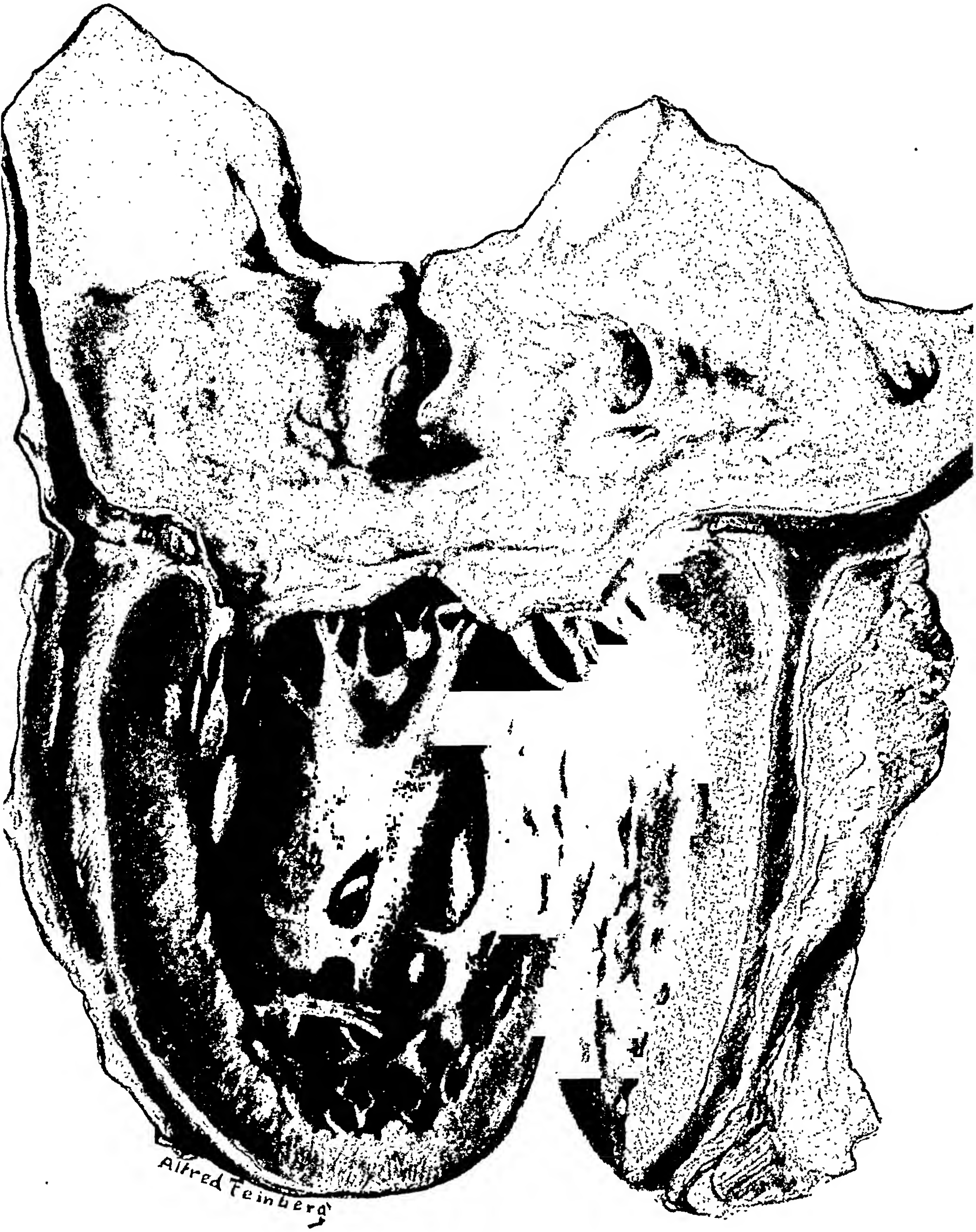

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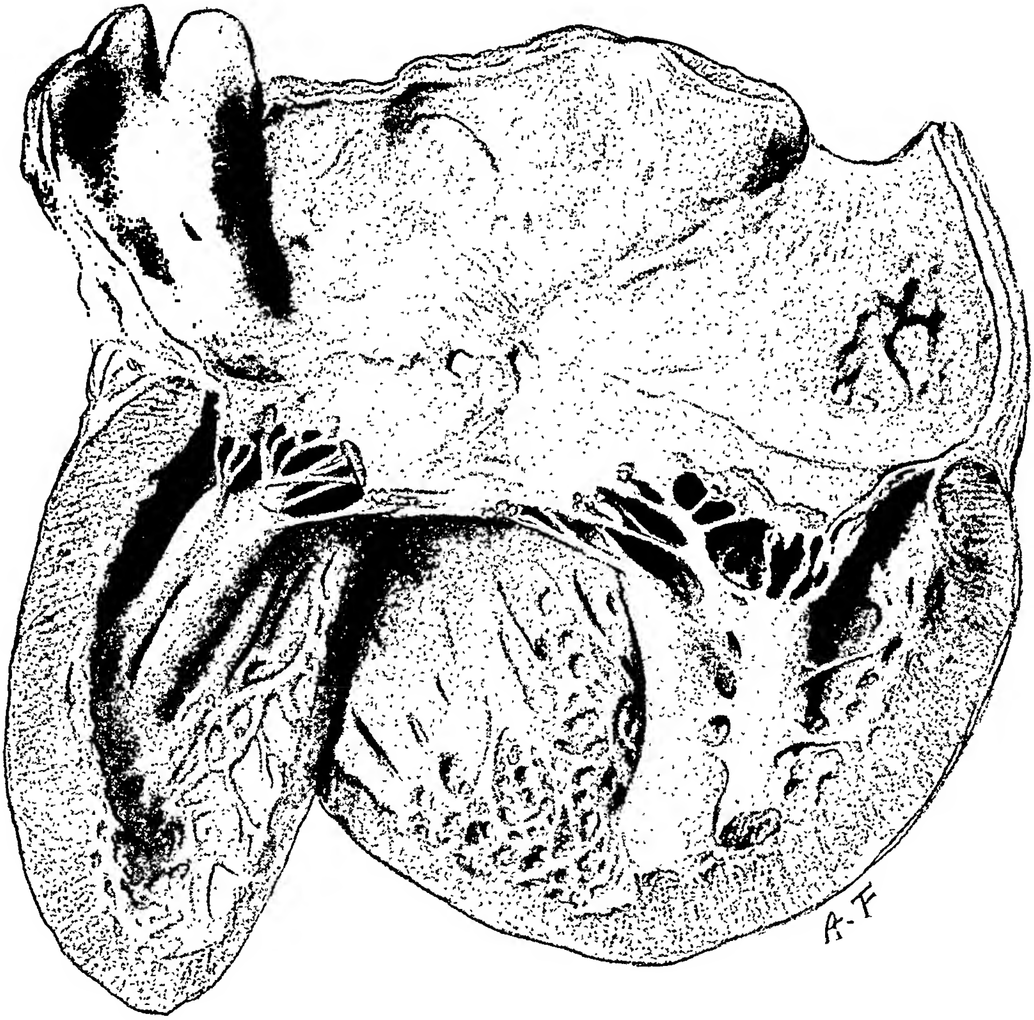

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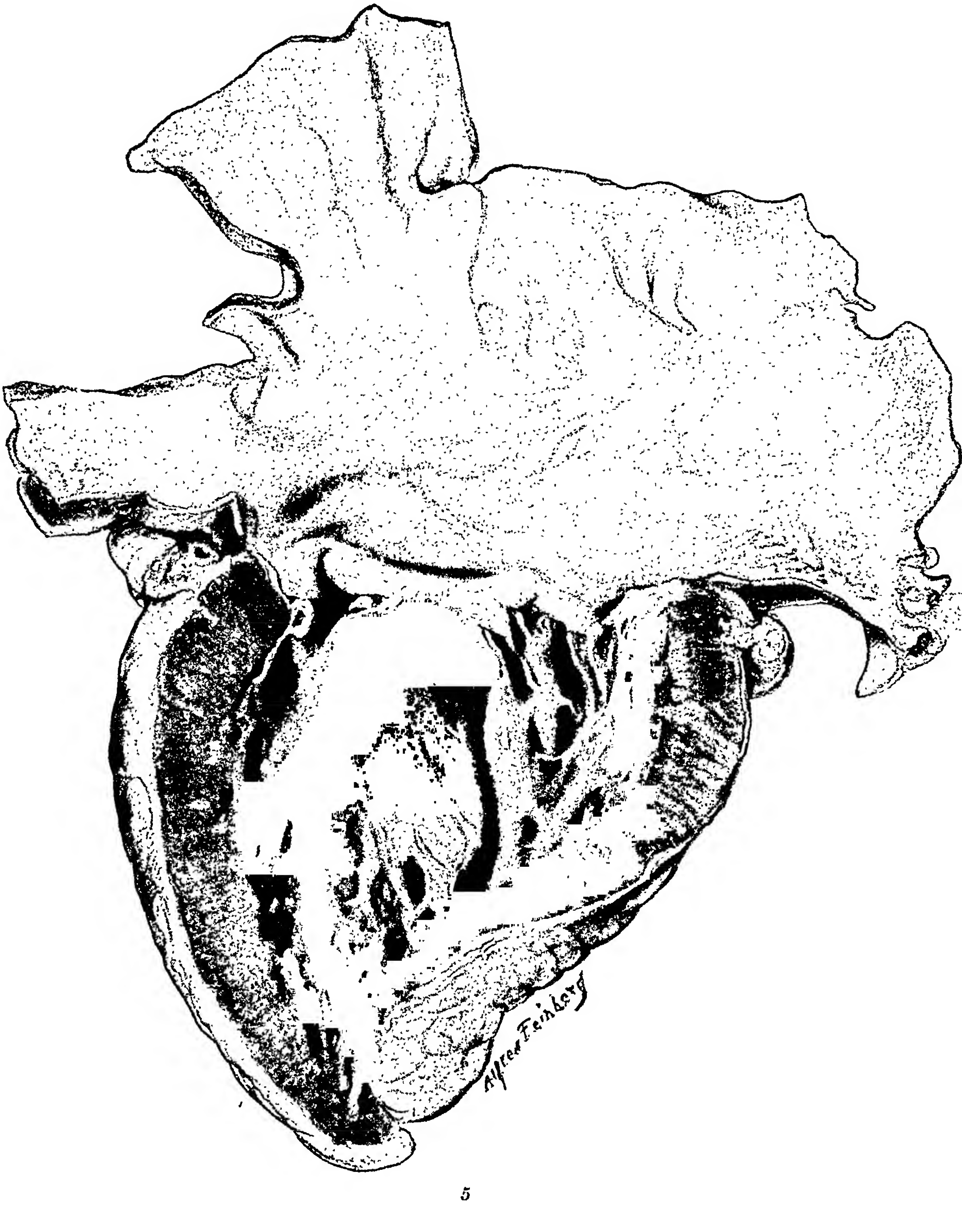

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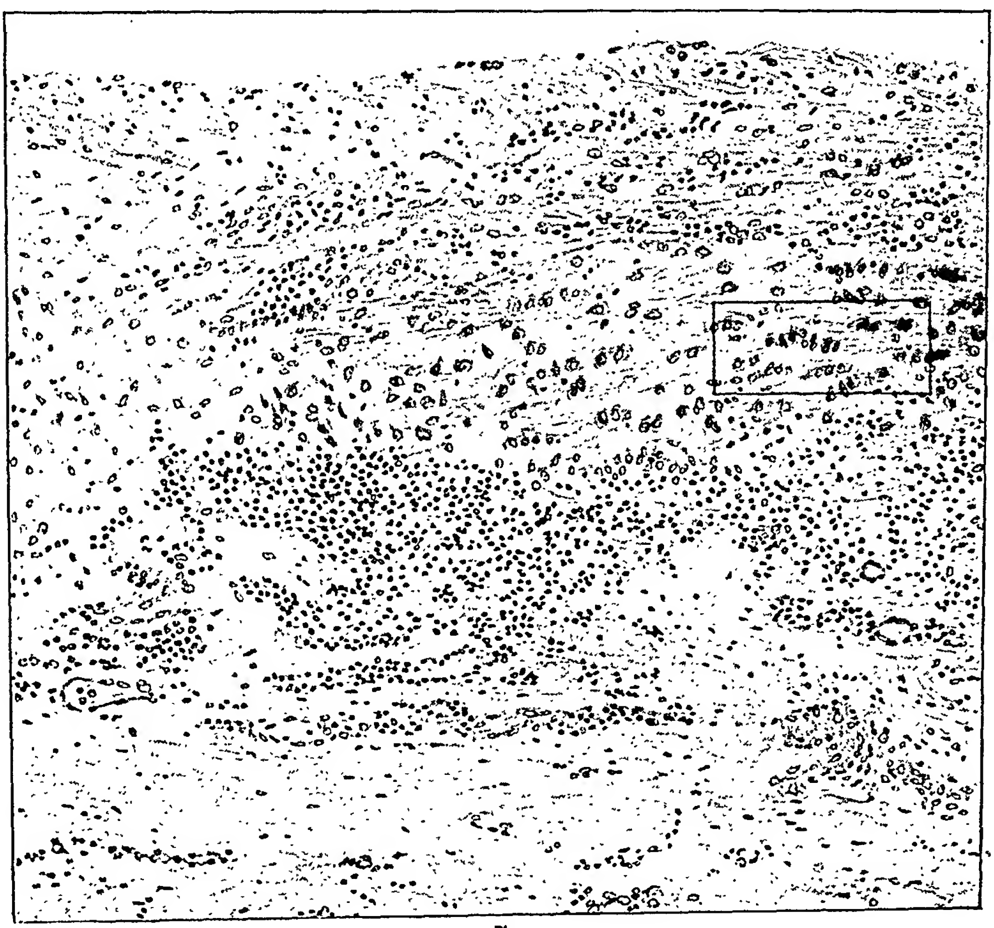
VonGlahn

Auricular Endocarditis of Rheumatic Origin

6



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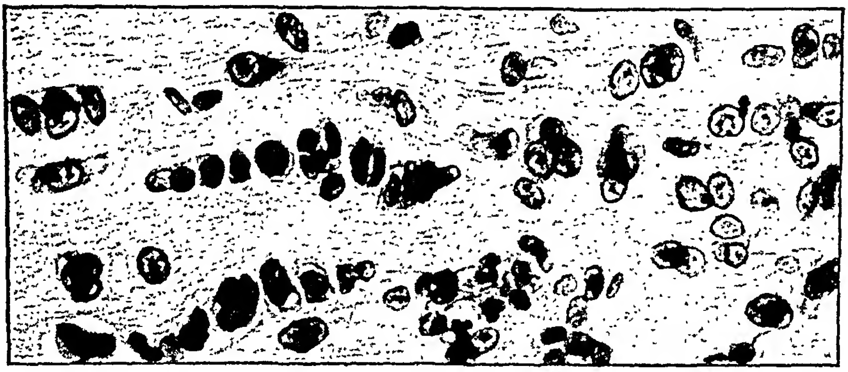

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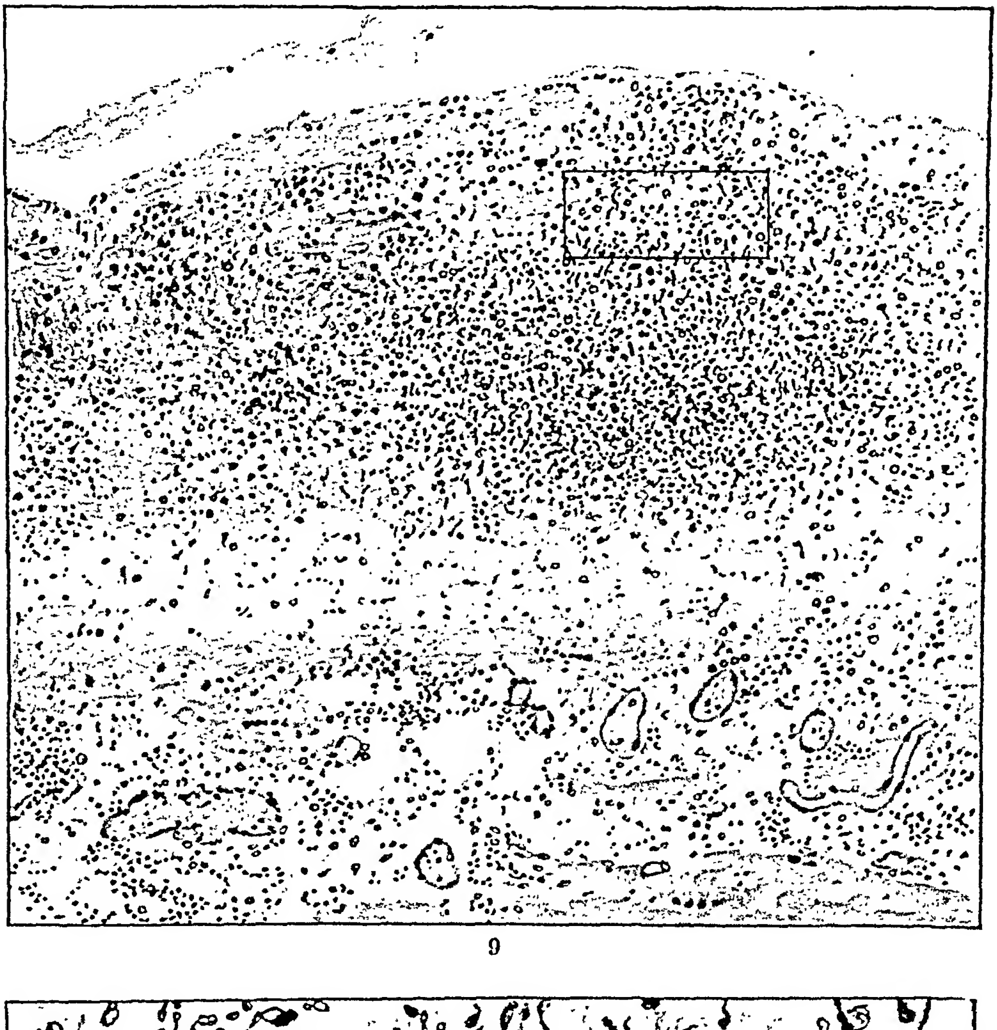

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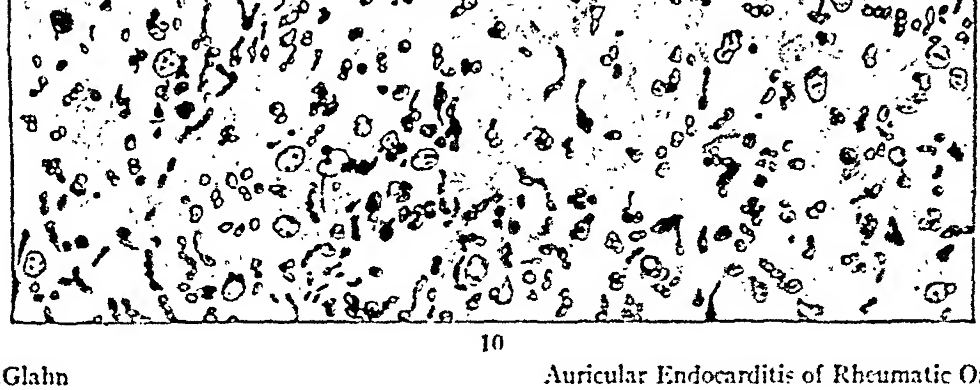

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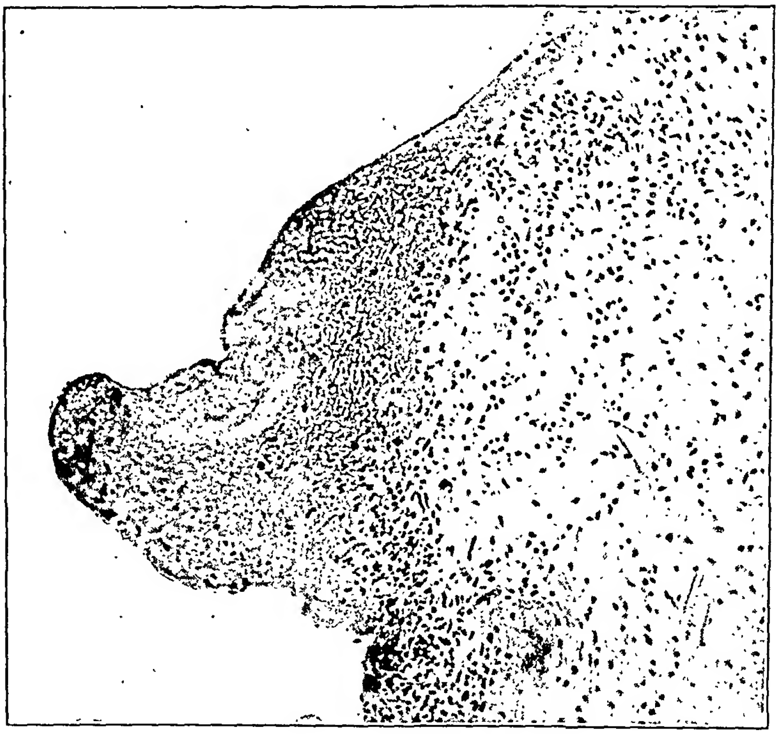

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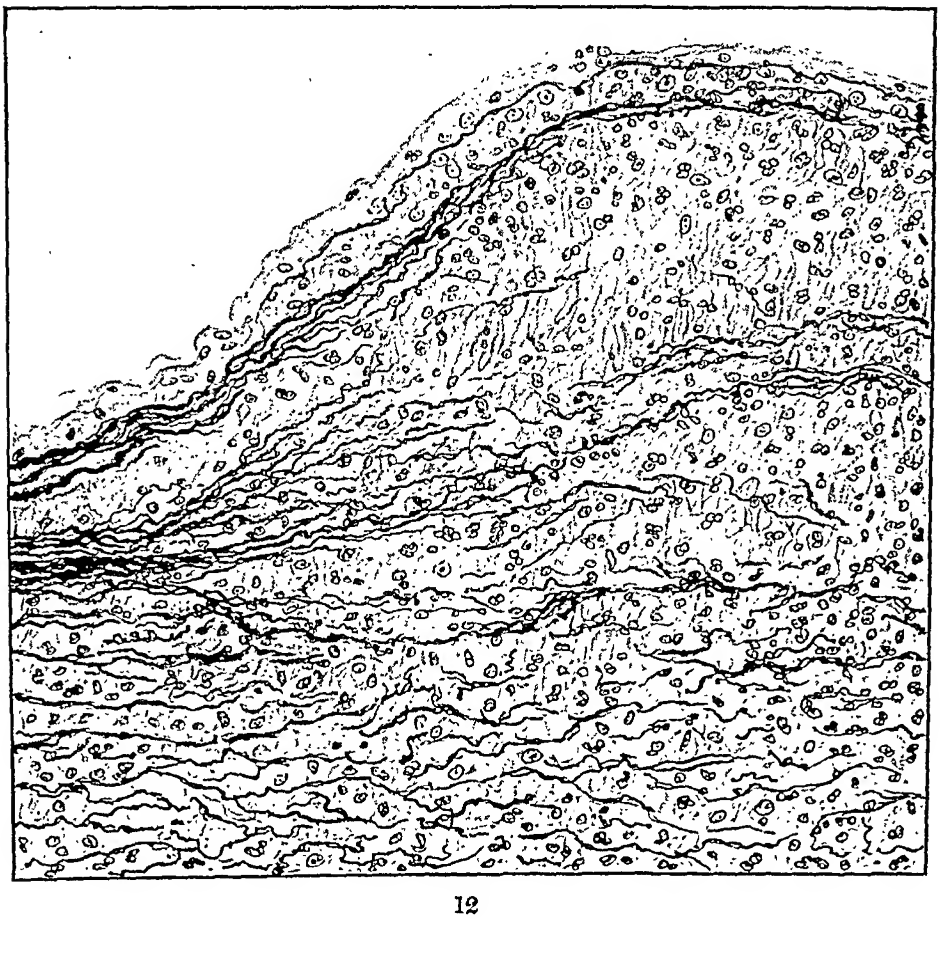

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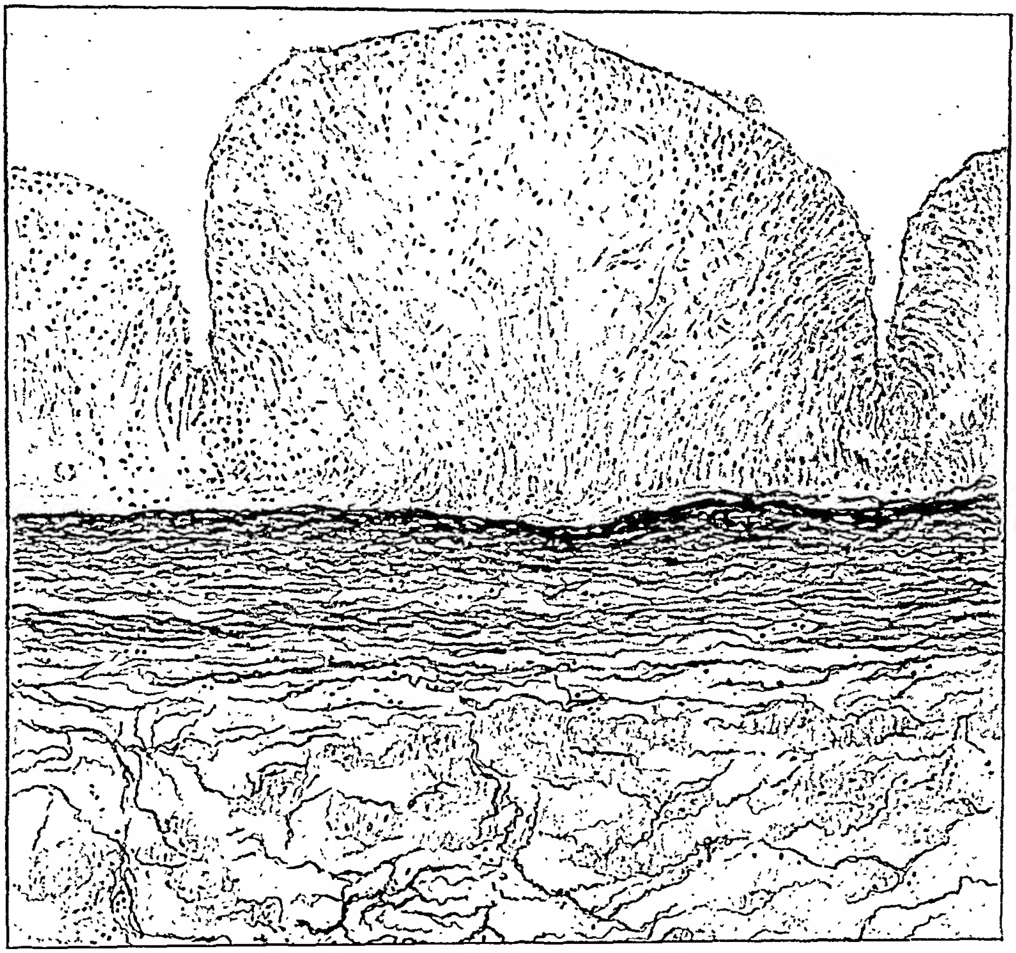

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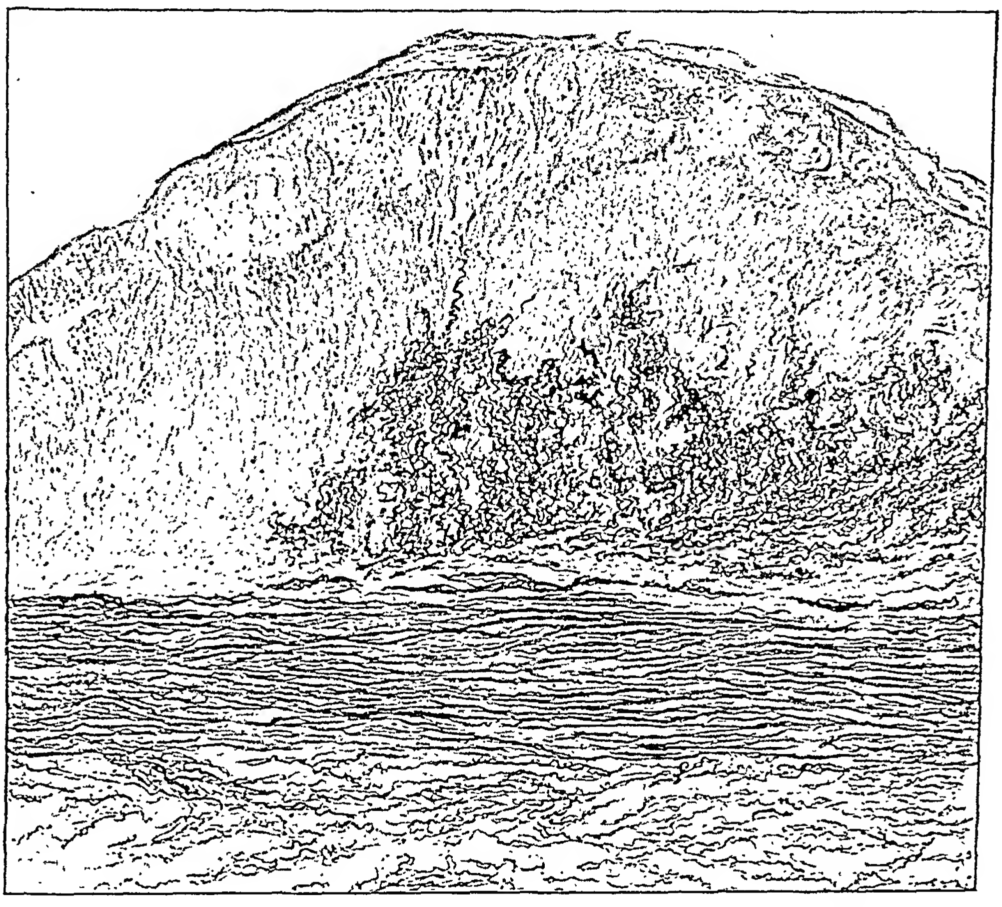

14

VonGlahn Auricular Endocarditis of Rheumatic Origin

A CASE OF RHEUMATIC AORTITIS WITH EARLY LESIONS IN THE MEDIA*

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and

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In a previous paper (1924)[1] dealing with the lesions of the aorta in rheumatic infections, two types of pathologic change were described as characteristic: the occurrence of Aschoff bodies or isolated Aschoff cells in the adventitia; and the presence of flame-shaped scars about the nutrient vessels of the media. In most of the cases examined, these medial cicatrices were dense and acellular, and were interpreted as healed or healing lesions. The material then available thus offered no opportunity to decipher the earlier changes leading up to the formation of the scars.

A case recently studied, in which the aortic lesions were acute and of unusual intensity, illustrates an earlier stage in the development of the medial alterations and is reported for that reason.

S. C., age 9, white, male (history 48682, autopsy 9627), was first admitted to the Presbyterian Hospital on Jan. 10, 1921, with painful joints in the arms and legs following exposure to cold three weeks previously. The course thereafter until his death on April 17, 1925, was typical of rheumatic fever with remissions, but with progressive involvement of the heart and pericardium. Blood cultures on his first admission were sterile.

The anatomic findings may be summarized as follows: *rheumatic endocarditis (mitral and aortic valves, and left auricle); rheumatic myocarditis; rheumatic pericarditis, organizing; serofibrinous pleurisy; cardiac hypertrophy and dilatation; chronic passive congestion of viscera; ascites.*

Only the aorta need be described minutely; grossly it shows nothing abnormal. Microscopically, however, striking changes are found, both in the adventitia and in the outer two-thirds of the media. The endothelial cells of the vasa vasorum are swollen. About these vessels are collections of small round cells and plasma cells together with many polymorphonuclear leucocytes. The lumen of some of the smaller vessels is filled with polymorphonuclears. In many parts

* Received for publication November 12, 1925.

of the adventitia, the cellular infiltration is diffuse and intense, with the polymorphonuclear leucocyte as the predominant cell.

In areas where the infiltration of wandering cells is less profuse, the connective tissue has taken on a peculiar appearance: the collagen bundles are swollen, and between them the connective tissue cells have somewhat the character of fibroblasts with basic staining cytoplasm and swollen nucleus. Multinucleated cells are not uncommon.

In none of the numerous blocks studied are there found definite Aschoff bodies.

The medial changes are essentially of the same character as those described in the adventitia, save that their distribution appears to be determined by the course of the nutrient vessels. The nutrient arterioles are thick walled, due in part to the swelling, and in some instances to the heaping up of the endothelium; in part also to the cellular infiltration and perhaps edema of their walls. About the nutrient vessels, pushing apart the adjacent elastic fibers, are profuse collections of cells, amongst which one can distinguish small lymphoid elements, polymorphonuclear leucocytes (often in considerable numbers) and larger elements with vesicular nuclei, prominent stellate nucleolus and strongly basophilic cytoplasm (Fig. 1). With the methyl green-pyronin stain, these larger cells react as do the Aschoff cells. Often these are aligned in rows between the elastic fibers (Fig. 2). A few multinucleated cells are seen.

The collagen fibers within and adjacent to the cellular infiltrations are swollen and more intensely stained with eosin or fuchsin than the normal collagen of the aorta. The elastic lamellae in these early cellular lesions are displaced, but not destroyed.

No bacteria could be demonstrated. Histologic study of the other tissues in this case, it should be stated, give no indication of a bacterial infection. The character of the valvular and myocardial disease is in all respects typical of rheumatic infection, and there are no embolic lesions.

A study of this case supports the view previously expressed that the perivascular medial scars are the sequel of an earlier focal perivascular inflammatory lesion.

We are indebted to Dr. Walter W. Palmer for permission to transcribe the clinical record, and to Mr. Alfred Feinberg for the drawings.

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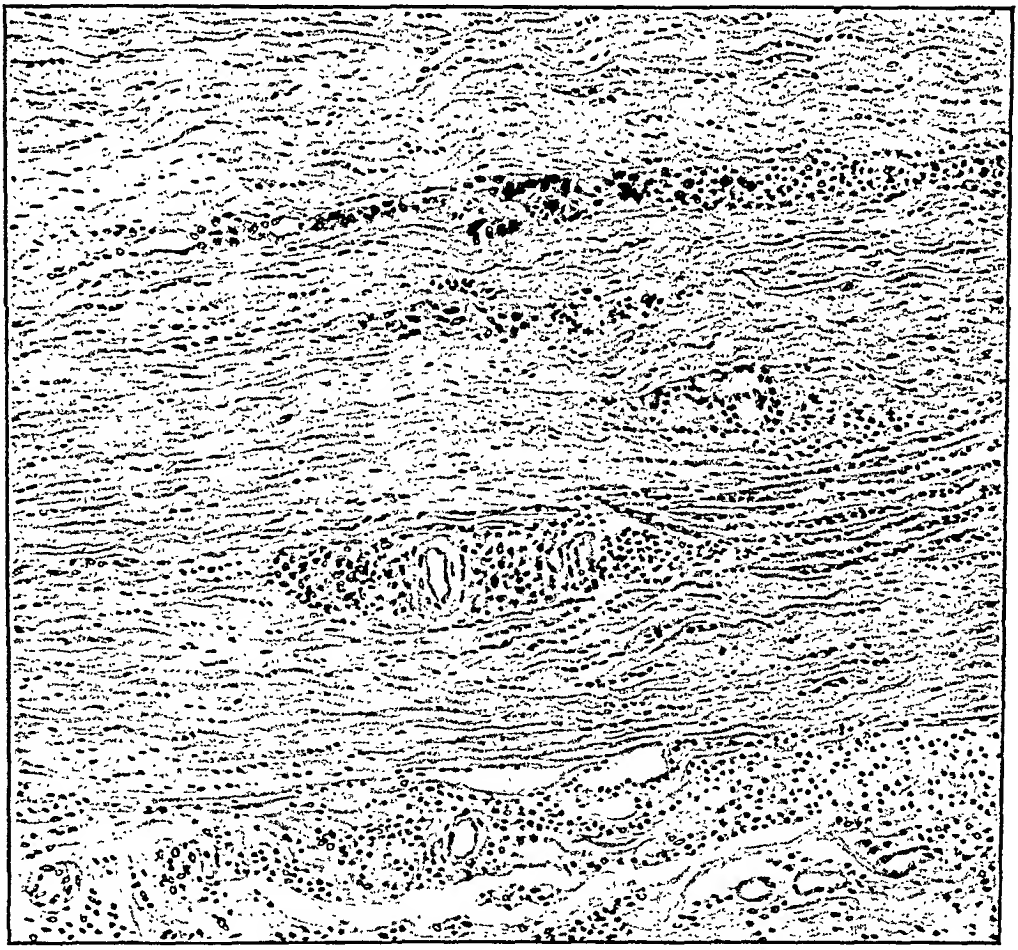
DESCRIPTION OF PLATE

Plate 10

Fig. 1. Acute rheumatic aortitis. Cellular infiltration about penetrating vessels in media.

Fig. 2. Acute rheumatic aortitis. Polymorphonuclear leucocytes and large cells with basophilic cytoplasm about penetrating vessels in media. Some of the large cells have multiple nuclei.



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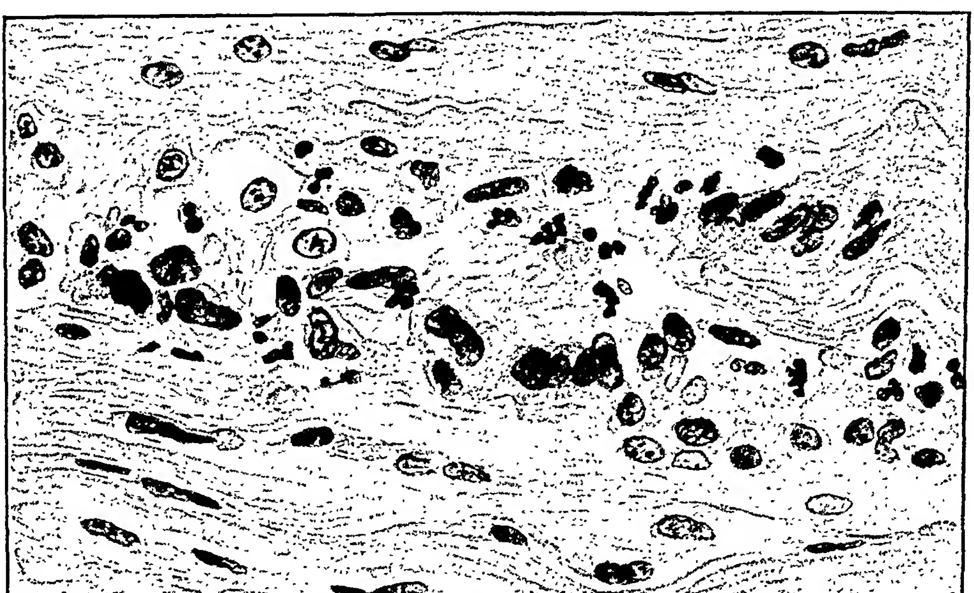

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Pappenheimer and VonGlahn

Rheumatic Aortitis

STUDIES ON COMPENSATORY HYPERTROPHY OF THE THYROID GLAND

VII. FURTHER INVESTIGATION OF THE INFLUENCE OF IODIN ON HYPERTROPHY OF THE THYROID GLAND WITH AN INTERPRETATION OF THE DIFFERENCES IN THE EFFECTS OF IODIN ON THE THYROID GLAND UNDER VARIOUS PATHOLOGIC CONDITIONS *

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In former investigations we have shown [1] in a relatively limited number of experiments that, contrary to the generally accepted view, administration of potassium iodid does not prevent compensatory hypertrophy in the thyroid gland of the guinea-pig. Considering the theoretical and practical importance of definite knowledge of the action of iodin on the thyroid under various conditions, we continued our investigations especially with a view of determining whether iodin may not only not prevent hypertrophy but also whether it may not even tend to increase it. We, therefore, carried out altogether seven series of experiments on 148 guinea-pigs, of which 72 received KI after previous removal of the greater part of the thyroid, and 76 served as controls. We also extended the time during which the iodin was allowed to act in some cases to approximately sixty days. Amounts of iodin varying in different experiments between 0.1 and 0.025 gm. KI were given each animal daily.

In order to summarize our results, it will be necessary to adopt a certain classification of the grades of hypertrophy. As we have done previously, we shall distinguish six types.[2] They are briefly as follows.

Grade I. Highest degree of hypertrophy. Entire liquefaction and absorption of colloid. Acinus cells high, cylindrical epithelium. Mitoses often present. Irregular acini; papillae present.

Grade II. Marked hypertrophy, similar to Grade I. Acini often form slits. In some peripheral acini, some pale soft colloid.

Grade III. Decided hypertrophy. Somewhat irregular acini; enlarged acinus cells, often granular, sometimes with mitoses. Colloid

* Received for publication Nov. 21, 1925.

diminished in quantity and consistency. Some acini form slits, but in the majority the colloid is merely diminished in quantity, softened, adherent and vacuolar. In a few acini the colloid may be more solid and retracted.

Grade III–IV. Acini slightly irregular; some with papillae, others regular in shape and lumen often wide. Acinus cells still moderately enlarged, sometimes with distinctly visible granules. There may be mitoses or amitoses. Usually colloid is distinctly softened, bluish, vacuolar, adherent or retracted; often somewhat diminished in quantity.

Grade IV. Very moderate hypertrophy. Usually regular, here and there some irregular acini. Walls of adjoining acini may be partially dissolved, resulting in the formation of spurs. Acinus cells somewhat enlarged, at least over wider areas. Mitoses rare. Colloid slightly softened, pale, bluish, usually vacuolar and attached to the epithelium. In some acini it may be more solid and retracted.

Grade IV–V. Trace of hypertrophy. On the whole, acini regular; acinus cells of medium size, but in places slightly larger. Colloid usually solid, retracted, but there may be some vacuolar, adherent colloid.

Grade V. Normal gland without hypertrophy. Regular acini, low to medium epithelium. Solid, slightly retracted colloid.

Grade VI. Acini generally very small; epithelium very flat. Colloid solid and slightly retracted. There is some indication that acini have been dissolved.

A COMPARISON BETWEEN THE EFFECTS OF THE EXTIRPATION OF THE GREATER PART OF THE THYROID GLAND ON THE COMPENSATORY HYPERTROPHY OF THE REMAINING GLAND TISSUE IN GUINEA-PIGS WHICH RECEIVED KI AND IN CONTROL GUINEA-PIGS

I and II KI Series.

I Series.* Twelve guinea-pigs were used. KI was injected subcutaneously; duration of individual experiment varied between seventeen and twenty-nine days. Average grade of hypertrophy was 3.54.

II Series.† Seven guinea-pigs were used. KI was fed by mouth; duration of

* This series was previously reported, *J. Med. Res.*, 1920, xli, 481. One case is omitted here, because the animal had not received an injection during the last ten days.

† This series was also previously reported; one guinea-pig included in the earlier report has been here omitted, because the remaining gland tissue was removed as early as fourteen days after operation.

individual experiments was twenty-four to twenty-five days. Average grade of hypertrophy was 2.8.

The average grade of these nineteen experiments combined was 3.21.

In these two series, the experiments were carried out during the winter as well as during the spring months.

The weight of the guinea-pigs varied between 400 and 800 gm.

The result of the individual experiments was as follows: Grade of 1.75 in 3 animals; 2.25 in 2 animals; 2.50 in 1 animal; 2.75 in 1 animal; 3.0 in 2 animals; 3.25 in 1 animal; 3.75 in 4 animals; 4.0 in 2 animals; 4.75 in 1 animal; 5.0 in 2 animals.

I Control Series. Twelve guinea-pigs may serve as controls for the animals of the I and II KI Series. The control animals were examined nineteen to twenty-five days following the operation. This series includes 9 animals of a first lot with an average grade of hypertrophy of 4.50, and 3 animals of a second lot with an average grade of 3.50. The total average grade was 4.06.

The weight was approximately the same as in the KI series, namely, 400 to 800 gm. Individual grades: 3.25 in 2 animals; 3.75 in 2 animals; 4.0 in 5 animals; 4.75 in 1 animal; 5.0 in 2 animals.

III KI Series.

Sixteen guinea-pigs. Duration of individual experiments varied between eighteen and thirty-one days. This series of experiments was carried out in the winter and spring. The average grade of hypertrophy was 3.69. The weight of the guinea-pigs varied.

Individual grades: 1.50 in 1 animal; 2.25 in 1 animal; 2.50 in 1 animal; 2.75 in 1 animal; 3.0 in 1 animal; 3.25 in 1 animal; 3.50 in 2 animals; 4.25 in 3 animals; 4.50 in 2 animals; 4.75 in 2 animals; 5.25 in 1 animal.

Nineteen experiments of the *II Control Series* correspond to the III KI Series. The duration of the individual experiments varied between nineteen and thirty-two days. The average grade of hypertrophy was 4.14. Individual grades: 1.50 in 1 animal; 3.0 in 2 animals; 3.25 in 5 animals; 4.0 in 1 animal; 4.25 in 2 animals; 4.75 in 3 animals; 5.0 in 5 animals.

IV KI Series.

Ten guinea-pigs were fed KI during a period which varied in nine animals between fifty-two and sixty-four days. In one animal the experiment was terminated thirty-one days after operation. The average grade of hypertrophy was 3.40. The individual grades were as follows: 2.50 in 1 animal; 3.0 in 3 animals; 3.25 in 1 animal; 3.50 in 1 animal; 3.75 in 2 animals; 4.25 in 2 animals.

Nineteen animals, one constituting subseries (*b*) of the *IV Control Series*, correspond to this IV KI Series. The duration of the individual experiments varied between thirty and sixty-five days. The average grade of hypertrophy was 4.36. The individual grades were as follows: 2.75 in 1 animal; 3.25 in 1 animal; 3.75 in 2 animals; 4.0 in 3 animals; 4.25 in 1 animal; 4.50 in 1 animal; 4.75 in 5 animals; 4.80 in 1 animal; 5.0 in 4 animals.

In fourteen of these nineteen animals, the duration of the experiments varied between forty-two and sixty-five days. In this subseries the average grade of hypertrophy was 4.54. The individual grades were as follows: 2.75 in 1 animal; 3.75 in 1 animal; 4.0 in 1 animal; 4.50 in 1 animal; 4.75 in 5 animals; 4.80 in 1 animal; 5.0 in 4 animals.

V KI Series (1922–23).

Altogether ten animals were fed KI in this series. The average weight of guinea-pigs varied between 400 and 600 gm. The duration of the individual experiments varied between thirty and thirty-two days. About one-fourth to one-fifth of one lobe was left at the operation. There were six experiments done during the summer months with an average grade of hypertrophy of 4.4 and four winter experiments with an average grade of hypertrophy 4.0. The total average was 4.25. The individual grades of the summer experiments were: 3.50, 3.75, 4.50 and 4.75, each in 1 animal; 5.0 in 2 animals. In the four winter experiments the individual grades were: 3.50 in 2 animals; 4.25 in 1 animal.

The following experiment may be cited as an example: guinea-pig 94, one lobe of thyroid completely removed and the greater part of the second lobe extirpated. Daily feeding of KI, 0.05 gm. Thirty days later animal killed by chloroform and examined. Weight of animal at time of operation was 564 gm.; at time of examination 607 gm. At necropsy about one-third of one lobe of thyroid but no isthmus was found. Microscopic examination shows acini to be irregular in outline; some acini are long drawn out. Through the solution and absorption of the walls separating neighboring acini some papillae are produced. The acinus cells are somewhat enlarged. The colloid is pale, partly vacuolar, partly absorbed; the quantity is reduced. Some acini are infiltrated with lymphocytes and some polymorphonuclear leucocytes and these cells contribute to the absorption of the colloid. The grade of hypertrophy was 3.50.

To this series corresponds the *VII Control Series* with twelve experiments. Eight of these were winter and four summer experiments. The duration of the individual experiments was thirty days. The average hypertrophy of the summer experiments was 4.4 and of the winter experiments 4.25. The total average was 4.3. The individual grades were: 3.25 in 1 animal; 3.75 in 2 animals; 4.0 in 2 animals; 4.25 in 1 animal; 4.50 in 1 animal; 4.75 in 1 animal; 5.0 in 4 animals. The grades in the four winter experiments were 3.75, 4.0, 4.25 and 5.0. In this series the difference between the KI Series and the Control Series is very slight.

VI KI Series. Winter, 1924 (January to March).

Eight experiments were carried out. Average weight of the guinea-pigs 400 to 600 gm. One-fourth to one-fifth of one lobe of the thyroid was left at the operation. Duration of individual experiments thirty to thirty-one days. At examination one-fourth to one-half lobe is found. Average grade of hypertrophy is 3.40. Individual grades: 2.25 in 1 animal; 2.75 in 1 animal; 3.0 in 1 animal; 3.25 in 2 animals; 3.75 in 1 animal; 4.75 in 2 animals.

The following is an example of a decided hypertrophy in a guinea-pig of the KI series: guinea-pig 453, male, 642 gm. initial weight

and 647 gm. end weight. Duration of experiment thirty-one days. At examination, one large and two small remnants, altogether more than one-third of a lobe was found. No isthmus. Microscopic examination: very irregular, large papillary acini with high epithelium. Some mitoses in acinus cells. Very much diminished, softened, partly liquefied colloid; in places it has entirely disappeared. In some acini the colloid is filled with cells; some phagocytes in acini. Some acini form only slits. Through degeneration (pyknosis) and solution, walls of acini disappear. Still some papillae are visible which are composed of cells with pyknotic nuclei and which are approaching solution. Large conglomerate acini with multiple papillae may develop. Grade of hypertrophy 2.25.

The *VIII Control Series* of seven animals corresponds to this series. The average grade of hypertrophy in this series was 4.21. The individual grades were as follows: 3.25 in 2 animals; 4.25 in 2 animals; 4.75 in 2 animals; 5.0 in 1 animal.

VII KI Series (June and July, 1924).

Nine experiments were made in which one-fourth to one-fifth of each lobe was left and subsequently 0.1 gm. KI was fed daily to each of five guinea-pigs and one-fourth of this amount to each of four other guinea-pigs. The initial weight of the guinea-pigs varied between 450 and 600 gm. The duration of each experiment was twenty-nine days. The average grade of hypertrophy was 4.27. The individual grades were as follows: 3.50 in 1 animal; 3.75 in 2 animals; 4.0 in 1 animal; 4.25 in 1 animal; 4.75 in 3 animals; 5.0 in 1 animal.

The findings in guinea-pig 52 (a female from 583 gm. initial weight to 664 gm. end weight; fed 0.025 gm. KI daily) were as follows: two remnants of thyroid were found twenty-nine days after operation; no isthmus. In piece "a" the acini show slight irregularities; some papillae are found; some large acini. The acinus cells and nuclei are hypertrophic. There are many mitoses. In these acini the colloid is soft, often vacuolar or partly dissolved. Some phagocytes in colloid take up some of the colloid. In places the colloid is somewhat retracted. The connective tissue is edematous. Piece "b" is on the whole similar. The acinus cells are here slightly enlarged, and the colloid vacuolar and soft. Frequent mitoses. Irregular acini. There are areas where the epithelium shows no enlargement and the colloid is solid, retracted. Grade of hypertrophy 3.75.

The *IX Control Series* corresponds to this KI Series. The time, duration of experiments, mode of operation and weight of animals were approximately the

same, but the guinea-pigs did not receive KI after the partial extirpation of the thyroid gland. Seven experiments were made; the average grade of hypertrophy was 4.80. The individual grades were as follows: 4.75 in 5 animals; 5.0 in 2 animals.

If we compare the average grade of hypertrophy in each of the seven KI series and control series, we find in each instance the average of the hypertrophy more marked in the KI series, except in the V Series where the six experiments of the KI series and the four summer experiments of the control series showed the same, relatively low grade of hypertrophy.

KI Series

I Series — 12 guinea-pigs; subcutaneous injection of KI; 17–29 days. Average grade of hypertrophy 3.54.

II Series — 7 guinea-pigs; KI fed by mouth; 24–25 days. Average grade of hypertrophy 2.8. Average grade *of Series I and II combined 3.21.*

III KI Series — 16 guinea-pigs; 18–31 days. Average grade of hypertrophy 3.69.

IV KI Series — 10 guinea-pigs; 52–64 days (1 animal 31 days). Average grade of hypertrophy 3.40.

V KI Series — 10 guinea-pigs; duration 30–32 days; 6 summer experiments, grade 4.4; and 4 winter experiments, grade 4. Total average of hypertrophy 4.25.

VI KI Series, winter, 1924 — 8 guinea-pigs. Duration 30–31 days. Average grade of hypertrophy 3.40.

VII KI Series, summer, 1924 — 9 guinea-pigs. Duration 29 days. Average grade of hypertrophy 4.27.

Control Series

I Control Series — 9 guinea-pigs; average grade 4.50. Three guinea-pigs; average grade 3.50. Average grade of both combined 4.06.

II Control Series — 19 guinea-pigs; 19–32 days. Average grade of hypertrophy 4.14..

Subseries "b" of IV Control Series — In 14 guinea-pigs average duration of experiment varied between 42 and 65 days. Average grade of hypertrophy 4.54.
In 5 additional animals of this series the duration of the experiment was above 30 days, but not as long as in the 14 animals. The average grade was 3.85.
The grade of both lots combined 4.36.

VII Control Series — 12 guinea-pigs; duration 30 days; 8 winter experiments; 4 summer experiments. Average grade of hypertrophy in summer experiments 4.4; in winter experiments 4.25. The average of both combined 4.3.

VIII Control Series — 7 guinea-pigs; same duration as KI Series. Average grade of hypertrophy 4.21.

IX Control Series — 7 guinea-pigs. Average grade of hypertrophy 4.80.

If we compare the average grade of hypertrophy of all the KI guinea-pigs with the average grade of all the control guinea-pigs, we find the following figures: Altogether seventy-two animals of the KI series lived until the completion of the experiments. The average grade of hypertrophy in these seventy-two animals is 3.65. Seventy-six guinea-pigs are included in the control series; their average grade of hypertrophy is 4.24. If we enlarge our number of control animals to 117 by including guinea-pigs in which the greater part of the thyroid gland had been removed, but in which no special substance was fed and which served as controls in other experiments, we find an average grade of hypertrophy of 4.13; this figure still differs distinctly from the figure applying to the KI series. This figure confirms the conclusion based on a comparison of the KI animals with their proper controls.

However, a consideration of the averages does not provide an entirely adequate basis of comparison of both series. For certain reasons of which we are at present ignorant, the grade of hypertrophy varies considerably in different individuals and within the same series. Furthermore, our grades are to some extent arbitrary and not expressing the differences between various animals in an entirely adequate manner. Thus the difference between 1.0 and 3.50 according to our grading is greater than the difference between 3.75 and 5.0; yet in certain respects the difference is perhaps greater between the two latter than between the two former animals. Five represents absence of hypertrophy. On the other hand, 1.0 and 3.50 merely signify different intensities of hypertrophy. It will therefore be necessary to supplement the average grades with the comparison of the number of animals showing the various grades of hypertrophy in the KI and in the control series.

GRADES OF HYPERTROPHY

		KI Series	Control Series
Grade:	1 — 2 (excl.)	4 guinea-pigs	1 guinea-pig
"	2 — 3 (excl.)	10 guinea-pigs	1 guinea-pig
"	3 — 4 (excl.)	29 guinea-pigs	19 guinea-pigs
"	4 — 5 (excl.)	22 guinea-pigs	37 guinea-pigs
"	5 or more	7 guinea-pigs	18 guinea-pigs

We can consider the grades 4.50 and higher as indicating lack of hypertrophy, the grades 3.50 (incl.) to 4.50 (excl.) as indicating a

mild degree of hypertrophy and the grades with a lower figure than 3.50 as indicating a very distinct and marked hypertrophy. We thus have three classes of animals representing different intensities of hypertrophy.

KI Series

Class I	(no hypertrophy)	: 19 guinea-pigs	= 26 per cent	= 26 per cent	
Class II	(mild hypertrophy)	: 27 guinea-pigs	= 38 per cent	with hypertrophy 74 per cent	
Class III	(marked hypertrophy)	: 26 guinea-pigs	= 36 per cent		

Control Series

Class I	38 guinea-pigs =	. . . .	50 per cent
Class II	23 guinea-pigs =	30.3 per cent	with hypertrophy 50 per cent
Class III	15 guinea-pigs =	19.7 per cent	

The comparison of the number of animals in the various classes in the KI series and control series shows a decided increase in the hypertrophy in the KI series over the control series.

Discussion

The results which we obtained in these experiments make definite the conclusion that potassium iodid does not have any inhibiting effect on the hypertrophy which develops in the guinea-pig in the remaining part of the thyroid gland after the greater part has been removed. On the contrary, they make it almost certain that KI intensifies the microscopic characteristics of hypertrophy during the period from the twentieth to the sixtieth day following extirpation. It increases the average size of the acinus cells and the number of mitoses; it promotes the liquefaction and absorption of the colloid; and tends to make the acini more irregular. As far as the macroscopic appearance is concerned, the remnants of the gland in the KI series are usually of good size.

We shall now attempt to correlate in a tentative way the principal facts established concerning the relations of iodin and the thyroid gland under pathologic conditions. In the guinea-pig we find that after extirpation of a great part of the thyroid, compensatory hypertrophy sets in in many cases and that this leads to a more or less far-going transformation in the structure of the gland. Thyroid feeding prevents hypertrophy and may perhaps even lead to a relative atrophy of the gland; feeding anterior lobe of the pituitary

also prevents the compensatory hypertrophy without apparently leading to an atrophy of the gland; the feeding of thymus has no noticeable effect; and the feeding of potassium iodid or the administration of iodin in some other form does not only not prevent or diminish the hypertrophy, but on the contrary tends probably to increase it.

However, we find that there are variable factors in the compensatory hypertrophy which are understood only very incompletely at the present time. A considerable number of guinea-pigs do not show these signs of hypertrophy which we find in other cases. Seasonal differences in weather seem to play a rôle in these variations, but they do not account for differences found in animals operated upon during the winter months. It may be that the time curve varies in different animals, and that in some of them the changes take a more rapid course than in others. In particular, it is thus possible that in control animals the hypertrophy takes a more rapid course than in the KI animals and that in the earlier stages which still have to be investigated, the relative preponderance of signs of hypertrophy is not, as at later periods, on the side of the KI guinea-pigs but rather on that of the controls. It may also be that in some animals a compensatory new formation of acini develops without the signs of hypertrophy occurring. However, the occurrence of mitoses in the acinus cells is usually associated with at least some hypertrophy of the gland cells. All these points need further elucidation before the problem of compensatory hypertrophy can be considered completely solved.

If we now turn to goiter in man, iodin seems to have different effects under different conditions. In children, small doses of iodin taken over a long period of time seem to prevent the endemic goiter, at least the kind that occurs in the Great Lakes region in this country. It also seems to cause the retrogression of developing goiter in young children; whether it has the same effect in Switzerland in the case of endemic goiter frequently associated with cretinism, needs still to be established. In the case of goiter of the adult, iodin does not seem to lead usually to a complete retrogression of the goiter. At most it seems to cause a temporary retrogression which is in many cases followed later by new growth. It has also been observed that a goiter which diminished in size as a result of a first administration of iodin, is in some cases found refractory to the

action of iodin if it is applied again at a later period. But in addition, iodin apparently causes in many cases a transformation of a simple goiter into a condition which, as far as structure of the gland and clinical symptoms are concerned, closely approaches Graves' disease. This transformation takes place especially in nodular goiters, but it may also occur in the case of the diffuse goiter.

In Graves' disease the administration of small doses of iodin seems to diminish the severity of the symptoms at least for a short time; it does not lead to a cure. As soon as the intake of iodin is interrupted the symptoms occur again. And even with long continued administration of iodin the symptoms seem to return after a transitory stage of improvement, although it has been stated by Fraser that a diminution in the doses of iodin may lead to a longer continued remission in the symptoms. In the case of toxic nodular adenoma of the thyroid gland, administration of iodin appears to aggravate the symptoms.

These seem to be the principal effects of the administration of iodin as far as they have been established at the present time. These various data appear in certain respects contradictory. The following attempt to interpret them can be regarded only as purely tentative and will have to be modified in accordance with the accumulation of additional experimental and clinical data.

The diminution of the specific substance (hormone) normally produced by the thyroid gland initiates changes in the gland, or perhaps primarily in the organism as a whole, which lead to hyperactivity and hypertrophy of the thyroid and thus tend gradually to counteract more and more through an increased production and discharge of hormone, the causes which led to hypertrophy. Thus, the lack of the specific hormone acts as a stimulus to the gland. If the hormone is administered directly the changes leading to thyroid hypertrophy are removed.[2] A somewhat related substance in the anterior pituitary gland acts in a similar, but not entirely identical way.[3] In order to undergo this compensatory hypertrophy iodin is needed and the gland tissue in process of hypertrophy has the ability to bind this substance and to use it for the building up of hormone and also of the hypertrophic gland cells. There is usually a sufficient amount of iodin taken in with the food to permit this restitutive process (hypertrophy) to proceed; but the optimal quantity does not seem to be present in many cases and therefore administration

of an additional amount of iodin increases the intensity of hypertrophy in many animals. Not before the organism has obtained a sufficient amount of hormone, does the stimulating mechanism come to a standstill. This point seems to be reached a long time before the remaining part of the thyroid has rebuilt the whole amount of thyroid removed. According to our experiments the hypertrophy of the thyroid gland in the guinea-pig is thus never complete, at least in the time during which we kept the animals under observation. Thus we may explain the difference which we found in the effect of thyroid or pituitary substance on the one hand and of potassium iodid on the other, the two former suppressing or inhibiting hypertrophy, while the latter on the contrary stimulates it.

If in infants and young children the amount of iodin necessary for the production of the hormone is lacking, growth processes set in in the thyroid gland, which are different from those characteristic of compensatory hypertrophy and Graves' disease, and are more nearly akin to those growth processes which lead to the embryonal development of the thyroid. Again we assume that not only in order to build up hormones, but also in order to undergo those specific growth processes consisting in hypertrophy of the acinus cells, combined with absorption of colloid, a certain minimum amount of iodin is required and that in the absence of this amount of iodin the growth processes occurring in the thyroid gland in response to the deficiency in hormone are of a different kind, consisting essentially of a new formation of acini, which are in a relative state of rest. The epithelium is therefore relatively low, the acini are regular and colloid is stored in solid form. This may be considered a sparing reaction on the part of the thyroid gland in which the small amount of hormone produced is stored in the acini where it can be slowly and gradually used, thus compensating for the diminished facilities for producing this necessary material for lack of which the gland suffers. On the other hand, in compensatory hypertrophy and in Graves' disease, the gland works with full vigor, gives off at once the hormone produced and thus, at least in the case of compensatory hypertrophy, it attempts to compensate for a deficient amount of substance by the increased intensity with which the remainder is used. Both of these reactions may therefore be considered as adaptations of the thyroid gland to the specific abnormal condition in which it is placed.

Furthermore, in compensatory hypertrophy the gland not only produces hormone in a relatively increased quantity, but it builds up increased amounts of thyroid tissue and thus gradually diminishes the need and with it the stimulus for increased activity. In contrast to this condition, the growth processes in the developing goiter in children cannot markedly lead to an increase in the production of hormone because the supply of iodin necessary for the building up of hormone is not provided.

If at this stage an additional amount of iodin is furnished the gland, it returns to its normal active state because the additional iodin removes the deficit in hormone and the gland can begin to function under adequate conditions, provided lack of iodin was the only or at least the principal factor in the causation of the goiter. If other important factors should contribute to or be mainly responsible for the development of goiter, it is conceivable that administration of iodin may not have the same restorative effect. But if this deficit in iodin continues for a long period of time, it is conceivable that changes gradually occur in the cell equilibrium which lead to abnormalities, in so far as (1) the growth of the gland may become irregular and certain parts may proliferate more than others, so that a nodular goiter develops in which again an increased amount of colloid may be stored with the consequent formation of a colloid goiter; and, (2) in addition, the equilibrium of the acinus cells changes in such a way that the reaction to iodin becomes different.

We know that under other conditions changes of a somewhat similar character may take place as a result of preceding stimulation in living organisms; a change perhaps somewhat akin to active cellular immunity or allergy. If after the developmental period of the thyroid has passed iodin is furnished to the gland, it no longer responds to it in the manner in which it did at an earlier stage. It has become either entirely or almost entirely refractory to iodin which is now no longer able to lead to the normal growth restriction as a result of adequate hormone production. In other words the gland has lost the ability to produce the normal amount of hormone even in the presence of iodin. In some cases these structural changes may perhaps be very slight or may be limited as yet to small areas so that they may escape recognition. The increased amount of hormone is given off in such cases in so large a quantity and so rapidly that toxic symptoms may develop. Similar is the effect

which has been observed after administration of iodin in cases of thyroid adenoma. The gland tissue is no longer under hormonic regulation to the same extent as is the normal gland or the early goitrous gland in children, a condition which bears some analogy to the behavior of tumors which also are only incompletely or not at all under hormonal or metabolic regulation on the part of the organism as a whole; while this is especially so in the case of malignant tumors, it is also found, although to a less extent, in the case of adenomas.

In the case of Graves' disease we have to deal also with a gland which apparently is no longer under the complete control of the organism. It over-provides the hormone and discharges the substance rapidly into the circulation. It thus behaves somewhat like a gland in compensatory hypertrophy, and the structure of the gland fully corresponds to this increased activity. We may, therefore, assume that in this condition also, the gland is in a stimulated state; but the character of the stimulation which leads to the increase of activity in the two cases may differ. It is unknown in the case of Graves' disease. In the latter disease, it is not due to absence of a sufficient quantity of thyroid tissue. While in compensatory hypertrophy the amount of active gland is much reduced and therefore toxic symptoms are lacking, in the case of Graves' disease the quantity of the active gland is not only not decreased, but increased, and thus toxic symptoms are produced. In both compensatory hypertrophy and Graves' disease there is the normal amount of iodin present in the organism and therefore the gland can undergo the same structural changes in both cases. Yet a relative lack of iodin may exist in Graves' disease, considering the increased amount of thyroid tissue and the excessive activity of the gland in this disease. Even in the case of compensatory hypertrophy, we found that administration of iodin in certain cases tended to increase the activity of the remaining gland, thus indicating a relative lack of iodin. We may, therefore, assume that in Graves' disease the optimal amount of iodin satisfactory for a hyperactive gland is not at hand; that this lack may either, in accordance with the suggestion of Plummer, lead to the formation of abnormal products which cause symptoms of intoxication, or may still further stimulate the gland to abnormal activity; and that the administration of iodin may remove at least some of the abnormal conditions under which the gland functions, thus diminishing temporarily the overactivity

of the gland. However, other interpretations of this effect are possible. At present it seems that no definite statement can be made as to the manner in which iodin diminishes the toxic symptoms in Graves' disease.

Conclusion

On the basis of a very large number of experiments it can be definitely stated that potassium iodid does not prevent or even diminish the hypertrophy of the thyroid gland in the guinea-pig, which follows extirpation of a great part of the thyroid gland. On the contrary, in all our series of experiments, the average of hypertrophy was greater in the animals which received potassium iodid than in the control animals. There are, in addition to the amount of thyroid removed and the amount of iodin fed to the animals, other variable factors which influence the degree of hypertrophy following extirpation, such as seasonal changes. As in former experiments, the average hypertrophy was less marked in the summer months than that in experiments carried out during the winter. There are other variable factors which influence the result, which are as yet unknown.

On the basis of these results a tentative explanation of the influence of iodin on the structure and function of the thyroid gland is given.

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CONCERNING "MALIGNANT THYMOMA"; WITH A REPORT ON A CASE OF PRIMARY CARCINOMA OF THE THYMUS *

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Introduction

Since reporting a case of malignant tumor of the thymus five years ago (Foot[1]), I have studied and reported two more; a fourth recently examined at necropsy in our department will serve as a basis for this paper and afford the opportunity for discussing malignant tumors of the thymus as a group. None of the four cases in this series exactly resembles any of the others, excepting that two were of the sarcomatous type, one being large-celled and the other small-celled. A review of this short series, which makes possible a critical and comparative study, has so changed my views on the histogenesis and classification of this type of neoplasm that a discussion of these seems desirable, as an attempt to clear up some of the confusion that exists concerning the "malignant thymoma." After reporting this fourth case, therefore, the entire subject will be considered at some length.

These tumors are distinctly rare, less than a hundred having been described to date. The epithelial type of thymoma is still rarer, Symmers and Vance [2] in 1921 stating that only four true primary carcinomas of the thymus had been reported prior to that time. Since then Foot and Harrington [3] and Jacobson [4] have each described one. As the recent average has been less than two cases of malignant thymic tumor reported annually, I have been unusually favored in being able to collect four cases in the last five years.

Case Report

Clinical Report. F. H., a white male, 45 years old, single and a gasfitter by occupation, was admitted to the Cincinnati General Hospital on four occasions between February 13 and May 30, 1925, when he was discharged dead.

Chief Complaint. Shortness of breath and pain in the chest, with cough.

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Present Illness. He felt well until nine weeks ago, when he began to have a dry cough which was brought on and intensified by stooping over. This attitude also caused dyspnea, weakness and dizziness. Shortly after the cough began he noticed swelling of his neck, most marked just after a fit of coughing, when his face became puffy. These symptoms increased until he was forced to prop himself up on a pillow in order to sleep in bed. He noted burning sensations and pain beneath the sternum, extending to the back, across the right chest and localizing between the scapulae. His appetite was good, his bowels regular and he had had no nausea or vomiting.

Past History. His general health had always been good. Aside from a history of chancroid and bubo nine years ago, his past history is irrelevant.

Family History. There was no history of malignant tumor in his family and no data were obtained that bear on this case.

Physical Examination. Negative, except for the chest and neck. There was engorgement of the veins over the right arm and in the right axilla. The respiratory movements were free and equal. The percussion note was impaired over the right upper chest, slightly so above and behind on the right. The breath-sounds were diminished over these areas. The neck was full; there was a firm mass beneath the right clavicle, extending upward and backward behind its outer half. The cricoid cartilage was at the level of the suprasternal notch. The thyroid was not palpable, but its isthmus seemed to impinge upon the examining finger during inspiration. There was no tracheal tug. The retroclavicular mass did not pulsate. Retrosternal dullness was markedly increased, being 8.5 cm. in the first interspace, 3.2 and 5.0 cm. in the second. Cardiac dullness was 2.5 cm. to the right, 12 cm. to the left; the point of maximum impulse was 10 cm. to the left in the fifth interspace.

Subsequent Course. The venous engorgement increased and the breath-sounds and percussion note became progressively impaired as time passed. Two weeks after admission, the patient complained of feeling a mass that rose and fell behind his sternum with each respiration. His cough persisted. He was given deep radiotherapy at the Cincinnati Jewish Hospital and, as the diagnosis of malignant mediastinal tumor was practically certain, he was told to continue this treatment once a week and to report frequently for observation. A skiagraph showed kyphoscoliosis and a large shadow in the mediastinum. Laboratory tests at this time were all negative.

On his first readmission the condition was found to be getting progressively worse; despite the weekly irradiation with deep X-rays there was more bulging in the right supraclavicular fossa and some had appeared in the left. His voice had become husky, but his cough was less troublesome. There was more fullness in the upper chest and neck and the mediastinal growth was unmistakably increasing. Nausea and vomiting were now added to the list of symptoms.

On his second readmission the patient remained only a day or two. In the two months that intervened since his first admission, the mass in the mediastinum had grown steadily and the superficial abdominal veins now shared in the engorgement hitherto noted over the upper chest and right arm. His cough had reappeared and he had developed an early secondary anemia.

On his third readmission he remained two weeks in the hospital and died about three and one-half months after the date of his first admission. His gastroenteric symptoms had increased; there was definite orthopnea; and the swelling in the neck had extended well upward beneath the right sterno-mastoid.

The superficial veins were now generally distended, particularly over the lower thorax. A wide area of retrosternal dullness was determined 4.5 cm. to the right and 5.5 cm. to the left in the fifth interspace. Dysphagia had developed. His pupils and pulses remained equal on both sides. Two weeks after his last admission, his cough which had been persistently dry and unproductive became productive and he raised large quantities of slaty, homogeneous sputum. Two days later, after feeling very ill for a day or so, he had a sudden profuse hemorrhage that gushed from his nose and mouth and he died within four or five minutes. During his final visit to the hospital his temperature, which had hitherto been essentially normal, became febrile and ranged from 99 to 102 F. Two skiagraphs showed that the tumor had increased until it had involved the entire upper lobe of the right lung. His treatment was, of necessity, symptomatic: deep X-ray therapy once a week and codeine to control his cough were the chief measures used.

NECROPSY. This was performed forty-five hours postmortem by Dr. H. A. Day, Resident in Pathology.

Anatomic Diagnoses: Carcinoma of the mediastinum with metastasis to both lungs, parietal pleura and to the peribronchial, supraclavicular and right axillary lymph nodes. Necrosis of the upper lobe of the right lung with fatal erosion into the right bronchus and its vessels. Extensive hemorrhage into right bronchus. Lobular pneumonia of right middle and lower lobes; also of the left lung, with extensive aspiration of fresh blood and possible hemorrhages. Healed fibrous pleuritis, right hydrothorax. Obliteration of the superior vena cava by ingrowth of the tumor. Brown atrophy of the heart and chronic passive congestion of the liver, spleen and kidneys. Healed cholecystitis and peritonitis. Fibrosis of the spleen and prostate. Subconjunctival hemorrhages, Residental caries and pyorrhea alveolaris.

Anatomic Findings: Body. That of a fairly well developed and somewhat emaciated white man apparently between fifty and sixty years of age. Body length 163 cm. Rigor mortis is universally present and there is slight postmortem lividity in the dependent portions. The hair is gray, the scalp rather bald. A bloody discharge escapes from the nose and mouth. The corneae are clear and the pupils unequal: right, 3 mm., left, 2 mm. in diameter. There are several petechial subconjunctival hemorrhages in both eyes. The mouth is befouled and coated with dried blood; the teeth are almost all gone, those remaining are mere snags and the gums are retracted and suppurating. The superficial lymph nodes are palpable as discrete nodules, excepting those of the right axilla and the supraclavicular group which are considerably enlarged and very firm. The extremities are all edematous, the right more so than the left.

Primary incision from the suprasternal notch to the mons pubis, with lateral subclavicular extensions, reveals very little subcutaneous fat. The peritoneal cavity is smooth, gray and blood-tinged and contains a few cubic centimeters of bloody, serous fluid. There are a few fibrous adhesions between the liver and diaphragm and between various intestinal coils, the colon and the gall bladder.

Thorax. Upon removing the sternum, a large mass is at once seen in the mediastinum, more or less adherent to the sternum and situated chiefly in the anterior mediastinum, where it surrounds the great vessels and adheres to the pleurae of both lungs. It is grayish, hard and composed of much fibrous tissue which cuts with a leathery resistance. It extends from just above the clavicles

to more than half way to the diaphragm and has included the mediastinal lymph nodes in its substance. These have a softer, yellower appearance quite different from that of the tumor, and several of them are necrotic and caseous. The necrosis resembles that of tuberculosis rather than that of tumor tissue. The neoplasm has invaded and completely occluded the superior vena cava and infiltrated the adventitia of the large arteries without penetrating their media. This infiltration has caused a definite constriction of the innominate artery, but the left subclavian and carotid do not seem to be much compressed although completely surrounded. The left pleural cavity is entirely obliterated by fibrous adhesions and is much thickened and apparently infiltrated by the tumor at its vault; the right pleural cavity is also obliterated in its upper portion by similar adhesions, but contains about 500 cc. of blood-tinged fluid in the lower. The heart is not remarkable.

The pleura of the right lung is covered with adhesions and dotted with gray tumor metastases which are more numerous over the upper lobe and somewhat umbilicated where they spread out beneath the pleura. On section, the upper lobe is found to contain a cavity 3×4 cm., in its approximate center; the walls of this cavity are necrotic and that of the right bronchus has been eroded and perforated just below the tracheal bifurcation. The tracheal and bronchial mucosa is eroded and congested in a zone 3 cm. in width, surrounding the perforation. Masses of firm, not yet necrotic tumor tissue lie in the walls of the pulmonary cavity and a few metastases are scattered throughout the rest of the lobe. The pulmonary tissue is also the seat of a patchy consolidation. It cuts with considerable resistance, is grayish and mottled with areas of congestion and anthracosis. The lower lobes are both congested and contain a few tumor nodules. The left lung is covered with shaggy, fibrous pleural adhesions throughout. On section it is found to contain numerous small areas of consolidation and congestion and a few minute tumor metastases. The bronchioles all contain fresh blood, probably aspirated.

The trachea is surrounded by the tumor as high as the suprasternal notch; its mucosa is edematous and congested. The bronchi contain fresh blood clots and the right shows the perforating erosion already described. The peribronchial lymph nodes are incorporated in the neoplasm and show anthracosis, caseation and a few areas suggesting metastases. The thyroid is not remarkable in gross, except for a small node in the anterior lower aspect of the right lobe. This later proves to be a parathyroid.

Abdomen. The abdominal organs show nothing relevant to this paper. The spleen weighs 190 gm., the liver 1655 gm. and the kidneys together weigh 315 gm. No extension of the tumor below the diaphragm is noted.

Microscopic Study. Diagnoses (N. C. F.): Primary carcinoma of the thymus with metastases to lungs, vessel walls and lymph nodes. Cardiac edema and increase in size of myocardial fibers. Low-grade lobular pneumonia with much organization of the exudate; interstitial pulmonary fibrosis. Infiltration of tracheal and aortic walls by the tumor. Fibrosis of the spleen; fibrous perisplenitis. Moderate toxic nephritis. Chronic fibrous prostatitis and slight subacute suppurative prostatitis.

Examination: The findings in the sections of various organs correspond to the above diagnoses and will not be dwelt upon, excepting where they have a direct bearing on the tumor. The lung sections show tumor metastases in which the neoplasm invades the alveoli, growing luxuriantly and producing innumer-

able Hassal's corpuscles. The wall of the trachea is infiltrated throughout by tumor tissue which has penetrated to the lumen and separated and destroyed the cartilage in so doing.

Microscopic Examination of the Tumor

Sections from the primary growth in the mediastinum are of comparatively little value for study, as there is so much necrosis that the tumor has been converted into dense masses of interlacing connective tissue interspersed with wide necrotic areas in which the outlines of many very large concentrically laminated bodies are evident (Fig. 1). There are wavy columns of young, well stained tumor cells at the periphery of necrotic areas. There is a widely distributed network of reticulum in this necrotic tissue. Undoubtedly the repeated irradiation with deep X-rays played a part in causing this breaking down of the tumor.

The neoplasm is best studied in its metastases to the lung and lymph nodes where there is far less necrosis and one can make out the morphology of the type cell. This corresponds to the thymic reticuloid cell and is about 10 to 17 microns wide and 13.5 to 34 microns long, according to its type of growth and stage of development (Figs. 2 and 3). The nucleus is elliptical and measures 6.5 to 13.5 microns in width and 10 to 17 microns in length. Three types of growth may be recognized: (*a*) solid, alveolar masses that resemble those of a medullary carcinoma, sometimes suggesting gland formation about rudimentary lumina; (*b*) anastomosing stellate cells with far less cytoplasm than the preceding type, forming alveoli of loosely reticulated structure; and (*c*) keratinized agglomerations of a few to many concentrically arranged cells identical in their appearance with Hassal's corpuscles.

The nuclei of the first two types are more or less vesicular, with a distinct chromatin network of delicate but rather deeply staining threads; one or more nucleoli are visible in each nucleus. The nuclei of the third type are usually rather pyknotic and degenerated. The cytoplasm of the tumor cells varies from scanty and vacuolated to very dense and refractile, resembling that of glandular epithelium and of keratinized dermal epithelium in the latter case.

The stroma is very fibrous and abundant in the primary growth and is composed chiefly of collagenous connective tissue; in the younger portions of the tumor and in the fresh metastases it is al-

most exclusively made up of reticulum. This surrounds the alveoli of the tumor without penetrating them (Fig. 4). The stellate type of tumor cell forms a reticulated matrix within the alveoli and this does not become impregnated with silver, being distinct from the true reticulum of the stroma and composed entirely of cytoplasmic processes. The pulmonary and lymphoid reticulum is very abundant and coarse in the vicinity of the metastatic cells, but it stops short at the margin of the tumor alveoli without penetrating them, just as would be true of an alveolar carcinoma.

To summarize, then: the tumor is of a definitely epithelial type; its cells correspond to those of a loosely built alveolar carcinoma, but differ from them inasmuch as they are stellate and more like embryonal thymic reticulum cells and also because they tend to form bodies that resemble thymic corpuscles more closely than the epithelial "pearls" of an epidermoid carcinoma.

The formation of these bodies indicates that they are, indeed, degeneration products. At first a few cells are observed lying singly and staining more deeply than their fellows. They are more compact, appear somewhat shrunken and their nuclei become increasingly pyknotic, elongated and narrowed (Fig. 5). Next, one finds several such cells grouped together and when a number have become cornified and clumped, they show a concentric arrangement (Figs. 6 and 7). Finally, the centers of these whorls undergo caseation. It is very evident that there are more of these bodies in the degenerated portions of the tumor and that the large necrotic foci contain innumerable shadowy Hassal's corpuscles; sometimes one of the smaller fields appears to be composed of a single, gigantic thymic corpuscle.

Giant cells and endothelial phagocytes are attracted to the necrotic areas in some cases, but the polymorphonuclear leucocytes, so common in the Hassal's bodies of the involuting thymus, are notably scarce. Eosinophiles and lymphocytes are not component parts of this tumor; it appears to be purely epithelial.

Discussion

Development of the Thymus. This organ arises from a paired primordium developing from the entoderm of the third pharyngeal pouches in the form of two tubular downgrowths. Schäfer (Jacob-

son cit.) considers that the fourth pair of pouches may share in the production of the gland, but Prentiss and Arey [5] point out that this portion of the primordium atrophies and contributes little thereto. The tubular processes soon lose their lumina and become solid cords which swell below and atrophy above, thus forming the two thymic lobes. The upper portions may give rise to accessory thymic organs. While entirely entodermal in man, the thymus of moles is ectodermal and that of pigs and fowls of mixed origin. In the human 50 mm. embryo the thymus still contains solid cords and vesicles of entodermal origin, but soon after this takes on its well known lymphoid appearance. The supporting tissue of the gland is formed of large, branching cells of entodermal origin, some of which become compacted and keratinized to form Hassal's corpuscles which characterize the organ histologically. Marine (Prentiss and Arey cit.) believes that they are the hyalinized and atrophic remains of entodermal vesicles.

While the reticuloid cells and Hassal's corpuscles are of undoubted entodermal origin, the lymphoid cells and the numerous eosinophile elements are of disputed parentage. Controversy has centered upon the lymphoid elements: are they differentiated entodermal cells, or are they lymphocytes of mesodermal origin, which have migrated into the thymic cortex from without? The adherents to the former theory constitute the "monistic school," to which belong Bell, Prenant, Schridde and formerly Pappenheimer.[6] Pappenheimer [7] has changed his views on this subject during the last few years, as have I. The second theory is championed by the "dualists": Dantchakoff,[8] Maximow [9,10] and Hammar [11,12,13] who has probably done more original work on this organ than has anyone, as is attested to by his article "Fünfzig Jahre Thymusforschung." [12] That these lymphoid cells are really lymphocytes was clearly indicated, if not proved, by the thymic tumor reported by Friedlander and myself [14] last winter (1925), in which the neoplasm was lymphoid in type and accompanied by an unmistakable lymphoid leukemia without any noticeable involvement of the lymph nodes in general. Fabian (Ewing cit.) has already collected a small series of cases that show the same or similar blood pictures. Thus it seems very probable that the "dualists" are warranted in their assumption that these are true lymphocytes and not differentiated entodermal cells.

The question as to whether the eosinophile elements of the thymus are of entodermal or of blood origin is not entirely relevant to this discussion; moreover it is far from being settled.

Literature. The average text-book on pathology passes by the malignant thymic tumors with rather cautious brevity, but Ewing[15] has discussed them at length in his book on neoplastic diseases. Rubaschow[16] compiled a list of thymic tumors including a compilation made by Hoffmann in 1896 and found that 52 sarcomas, 12 carcinomas and 11 miscellaneous tumors had been described up to that time (1911). The reader will find these earlier cases listed in Rubaschow's excellent article. If we exclude the miscellaneous tumors and add those reported since 1911, a total of 81 reports will be obtained. Symmers and Vance[2] state that only four true primary malignant epithelial tumors have been reported up to the time they published their paper; since then Jacobson[4] has described one, Foot and Harrington[3] another of rather mixed type, and the tumor now under discussion is the seventh.

Briefly listing the articles dealing with this subject, there are two excellent ones by Ewing[17] and Vanzetti,[18] and other papers by Wiesel,[19] Barbano,[20] Roccavilla,[21] Simmonds,[22] Speed,[23] Perrero,[24] Letulle,[25] Chiari,[26] Paviot and Gerest,[27] Thiroloix and Debre,[28] Cleland and Beare,[29] Harvier,[30] Sweany[31] and Helvestine.[32] Some of these reports include more than one malignant tumor of the thymus.

Diagnosis of the Thymic Origin of these Tumors. It seems to be agreed that the following characteristics point to the thymic origin of a given tumor:

1. The growth is found in the anterior mediastinum at the site of the thymus and is firm and not very lobulated.
2. It does not tend to invade bone, extending down the sternum without involving it. The tumors reported by Symmers and Vance and by Jacobson both invaded vertebrae and constitute exceptions to this rule.
3. The pleura and pericardium are involved by direct extension, rather than by metastasis, and the lung may also be directly invaded.
4. There is a general resemblance of the type cells to those of the embryonic or post-embryonic thymus and the presence of structures resembling Hassal's corpuscles while helpful, is not essential to diagnosis.

5. The sarcomatous tumors occur in children or young adults, the carcinomatous in patients of "cancer age," in later life.
6. Metastasis below the diaphragm is very rare, being reported in only three cases: that of Ambrosini,[33] in Symmers and Vance's second case and in the case described by Friedlander and myself.

There is a good deal of dogmatic discussion in the earlier papers that is tending to give way to a more liberal interpretation of these tumors as our experience increases.

Special Consideration of this Case. The interesting features of the case we are discussing are: (*a*) It is definitely primary in the thymus and shows an unusually marked production of Hassal's bodies, not in the way Jacobson describes it in connection with his tumor, *i. e.*, by proliferation of "Hassal's corpuscle cells," but rather by a process of degeneration somewhat similar to that seen in the involuting thymus.* Ewing has already called attention to the similarity between this normal process and that seen in epithelial thymic tumors. (*b*) The invasion of the superior vena cava corresponds to the same feature noted by Letulle in his second case and, in a measure, to that in the inferior vena cava described in my third case (Friedlander and Foot[14]). This definitely indicates a transportation of tumor cells by the blood stream in the case of malignant thymic tumors and makes one wonder why metastases at a distance from these growths are so uncommon. (*c*) Another strange fact noted in this tumor is the comparative absence of true reticulum from its parenchyma, whereas reticulin fibers were abundant in the other tumors of my series, particularly the third. It was most abundant in the frank lymphosarcoma. If the thymic "reticulum cells" form the reticulum of the thymus, why should one find practically no reticulum in a tumor composed largely of cells that correspond very closely in their morphology with the embryonal "reticulum cells" and abundant reticulum in a growth whose type cell closely resembles the ordinary microlymphocyte? This point is worthy of further consideration.

* Since this paper was submitted for publication, H. L. Jaffé and Alexandra Plavska (*Proc. Soc. Exp. Biol. and Med.*, 1925, xxii, 91; and *Proc. Am. Soc. Exp. Path.* (to be published) have reported investigations on the transplanted thymus of the guinea-pig that tend to confirm the assumption that Hassal's corpuscles are degenerative products of the thymic reticuloid cells and are unconnected with the remnants of the epithelial ducts of Remak, or thymic vesicles.

Classification of Malignant Thymic Tumors in General. We are not interested here in the benign growths of this organ and shall consider only the malignant variety. The term "thymoma" applied to these tumors is significant. In naming a tumor one does not add "oma" to the stem designating an organ without implying a certain hesitancy as to the histogenesis of the growth; otherwise one would call it by a more specific name. This is particularly true in the case of thymic tumors. Because of the uncertainty that prevailed in connection with the histogenesis of the various cellular elements of the organ, one hesitated to call the tumors obviously originating in its cortex "lymphosarcomas" until the origin of the lymphoid cortical cell was established.

With three types of cell composing the organ one could, theoretically, expect tumors of epithelial, lymphoid and eosinophilic types, as well as a mixture of these. Several primary epithelial malignant growths have been described: typical adenocarcinomas; tumors resembling epidermoid carcinomas and containing more or less well developed Hassal's bodies, or "pearls"; and new growths in which the type cell approximates the entodermal thymic reticulum cell, with or without rudimentary Hassal's bodies. Carcinomas originating elsewhere and metastasizing to the thymus, or its rest, are not truly thymic and should be excluded from this category. The majority of malignant thymic tumors, however, have been of the general type of the lymphosarcoma with cells varying from large, round or ovoid bodies to small forms closely resembling microlymphocytes. The first and third tumors of my small series typify these two. The eosinophile type, which we have stated as an hypothetical possibility, has never been described so far as I know, and it does not seem likely that it ever will be if these cells be true blood eosinophiles. Ewing has noted the abundance of eosinophiles in certain thymic sarcomas and has drawn attention to the similarity of these to Hodgkin's granuloma. The fourth possibility, a tumor of mixed type, is already on record. Hare and Rolleston (Jacobson cit.) have described a dermoid cyst and a mixed tumor; and Harrington and I reported a malignant thymic tumor in which epithelial ducts and cysts alternated with large fields composed of thymic reticulum cells, interpreting the origin of both types as entodermal.

Let us consider the probable origin of the various types just enumerated. The sarcomatous is probably derived from the meso-

blastic elements that invade the thymus during its early development. The reason for this statement has already been sufficiently discussed. One must be on the alert, however, for types of thymic tumor that resemble the large spindle cell fibrosarcoma but are, in reality, made up of the entodermal reticulum cells and are therefore of epithelial nature. True fibrosarcoma might, indeed, arise from the fibrous perivascular tissue of the thymus; but we do not find it described thus far. Very often there will be a sufficient number of rudimentary Hassal's corpuscles to indicate entodermal origin in doubtful cases.

The epithelial tumors originating from entodermal tissue in the thymus are sufficiently definite in their type to render a diagnosis fairly simple. They may be composed of glandular complexes, alveoli or concentrically arranged masses of squamous cells identical in appearance with the typical thymic corpuscle. They probably originate from the entodermal vesicles of the organ and from their duct-like derivatives. Ewing has indicated that there may be some connection between tumors of this type and a disturbance in the normal thymic involution.

The resemblance between the mixed type of epithelial "thymoma" and some forms of mediastinal teratoma is striking and it may be that such a tumor as that described by Harrington and myself is, therefore, to be considered an embryoma, or unripe teratoma. For a discussion of the mediastinal teratomas the reader is referred to papers by Smith and Stone[34] and Böhmig.[35] Our tumor, however, could be sufficiently explained by a persistence of those epithelial elements which differentiate to form the thymic "reticulum" and the Hassal's corpuscles; cells originating in the primitive thymic entodermal vesicles and hence not totipotential (in the sense of the term as used by Adami) as are the parent cells of the teratoma and its malignant prototype, the embryoma. The tumor we described is apparently very uncommon, as none of the thymic tumors hitherto described exactly corresponds with it. It occurred in a young child and bears the same relation to the other thymic epithelial tumors that malignant epithelial growths in children bear to carcinomas in individuals of advanced age.

In closing, it is interesting to note the almost complete necrosis of the tumor reported in this article, a fact that is in all probability due to the prolonged treatment with deep radiotherapy. Groover,

Christie and Coe[36] have reported two cases in which thymic tumor was diagnosed clinically (but not proved pathologically) and in which deep radiation resulted in a marked remission in the tumor in one case and an apparently complete and permanent disappearance in another. Both of these occurred in adults, but it is impossible to tell what type of thymic tumor they represented.

A complete understanding of malignant thymic tumors will, of course, never be arrived at until we know more about the thymus and until the question of the etiology of malignant tumors in general has been answered. In the meantime, we may assist investigators of both these questions by carefully studying and reporting every case of "malignant thymoma"; for the study of teratology has already accumulated much evidence which is of distinct value to the embryologist and histologist, as well as to the science of medicine.

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DESCRIPTION OF PLATES

PLATES 11–14

FIG. 1. A portion of the mediastinal tumor showing necrosis at bottom and a large Hassal's corpuscle at top; separating them is a mass of loose connective tissue. x 500.

FIG. 2. Metastasis to the lung, tumor growing in the air sacs. A mass of necrotic tissue with two Hassal's bodies (or two portions of a partially necrotic one) is seen in one corner. x 500.

FIG. 3. Metastasis to a lymph node; note the duct-like grouping of some of the cells, the hyalinized masses of concentrically arranged cells and the loose reticulum of tumor cells. x 500.

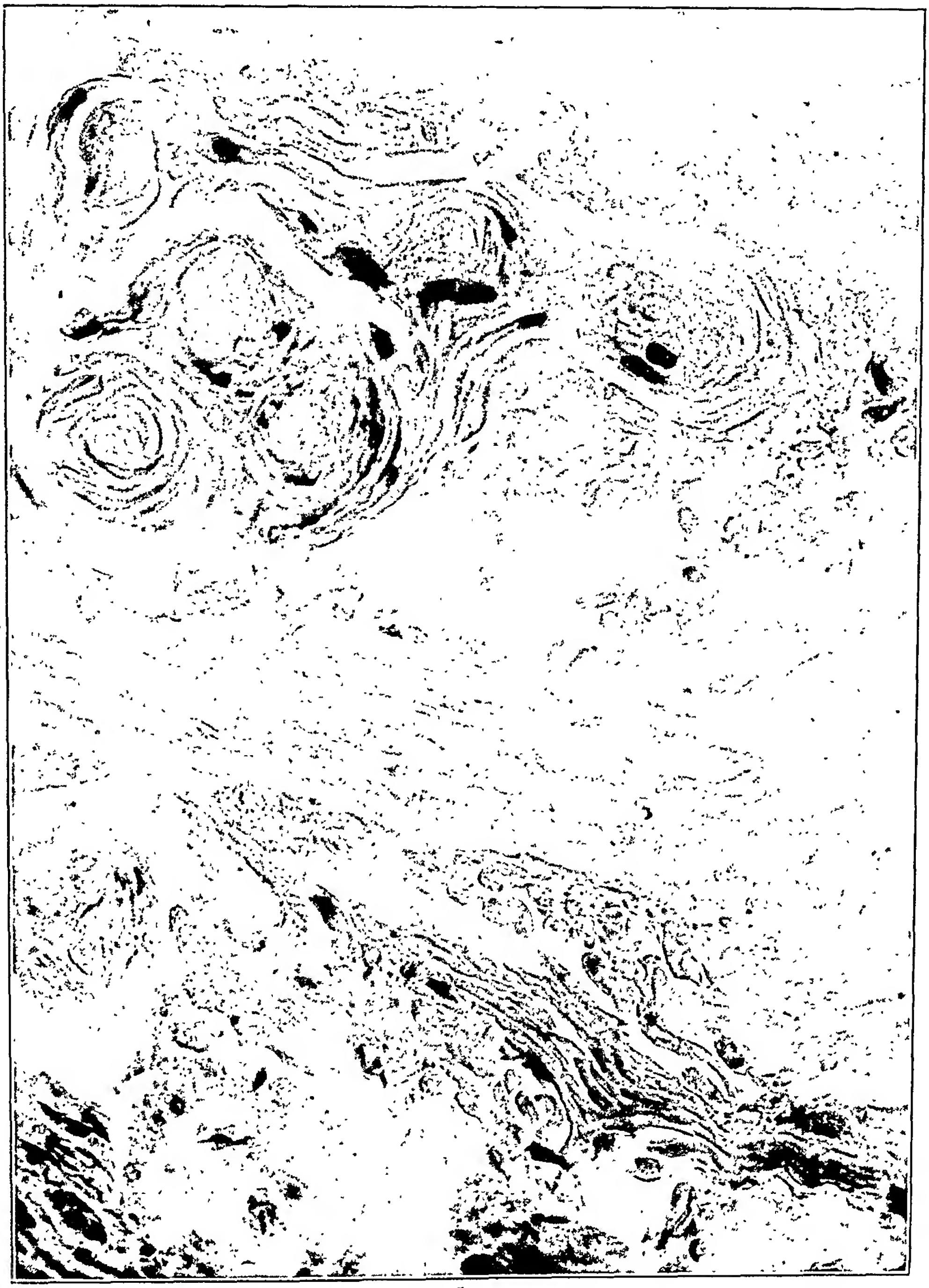
FIG. 4. Metastasis to the lung showing that the only true reticulum is that of the pulmonary alveolar framework. The very fine threads connecting various tumor cells are cytoplasmic processes, not true fibers. x 500.

FIG. 5. The beginnings of a Hassal's corpuscle; two hyalinized cells at the center of the picture represent this stage. x 1,000.

FIG. 6. Somewhat larger group of hyalinized cells, the nuclei becoming pyknotic. Probably intermediate between the preceding and following pictures. x 1,000.

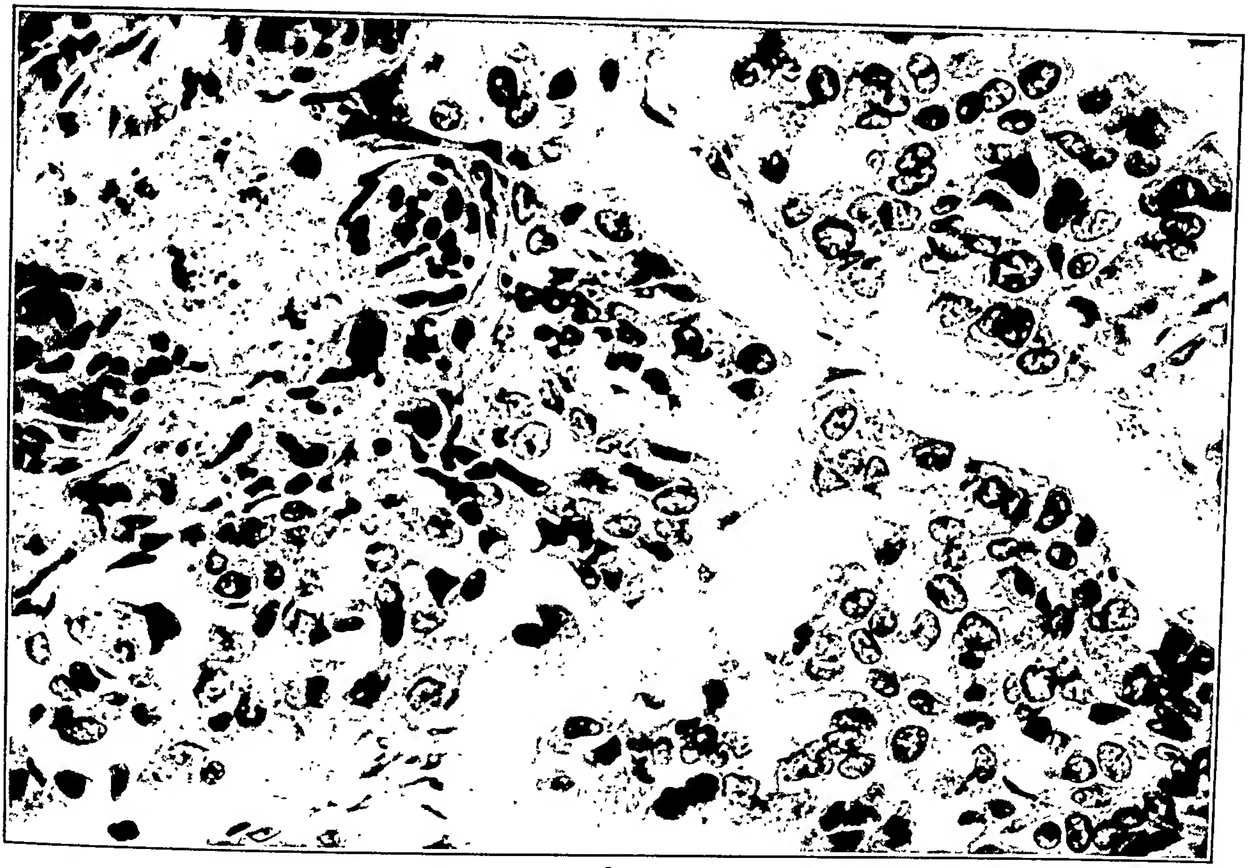
FIG. 7. Portions of two completed Hassal's corpuscles with beginning hyalinization, or at least much increased density, of the cytoplasm of a large field of tumor cells. Other tumor cells retain their faint, spidery outlines. x 1,000.

All the sections from which these photomicrographs were taken were fixed in Zenker's fluid, cut at five microns and stained with Harris' hematoxylin and eosin, except that for Fig. 4 which was impregnated with silver and counterstained with the Van Gieson method to demonstrate reticulum.



1

Foot Malignant Thymoma



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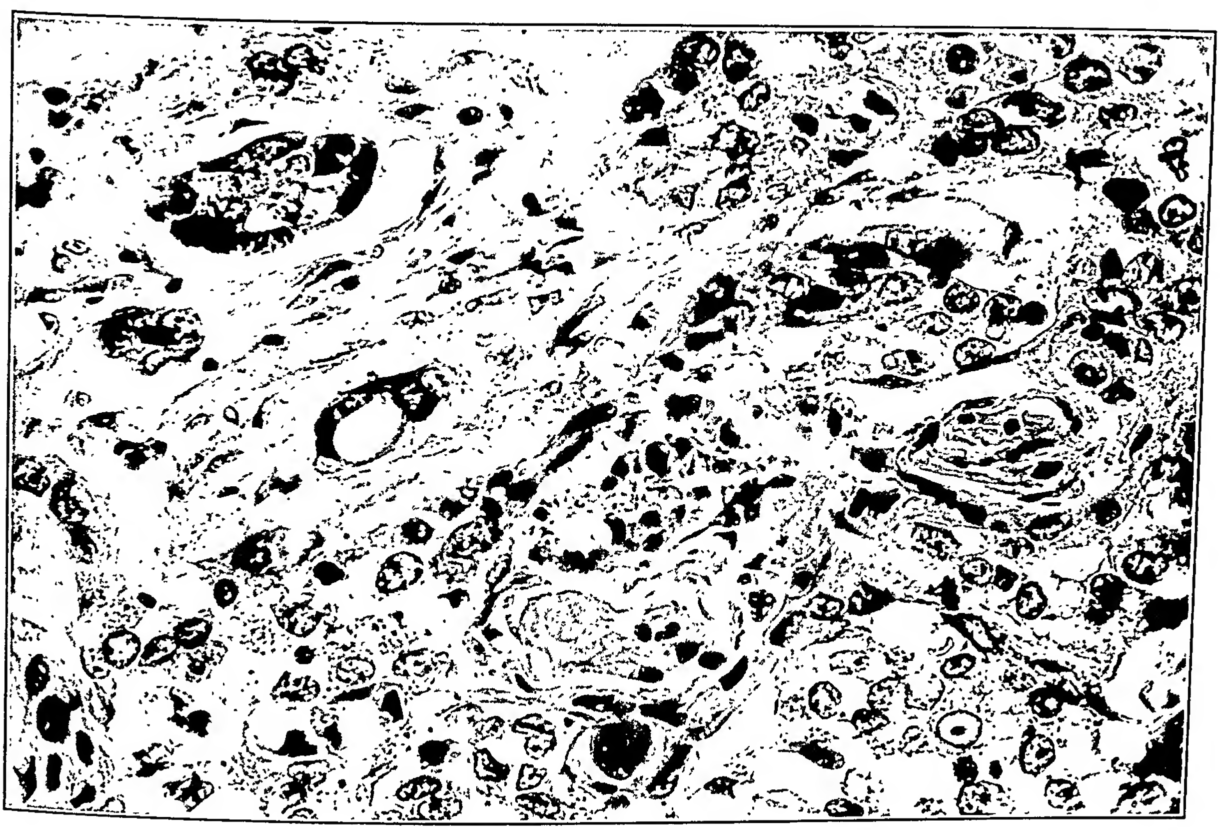

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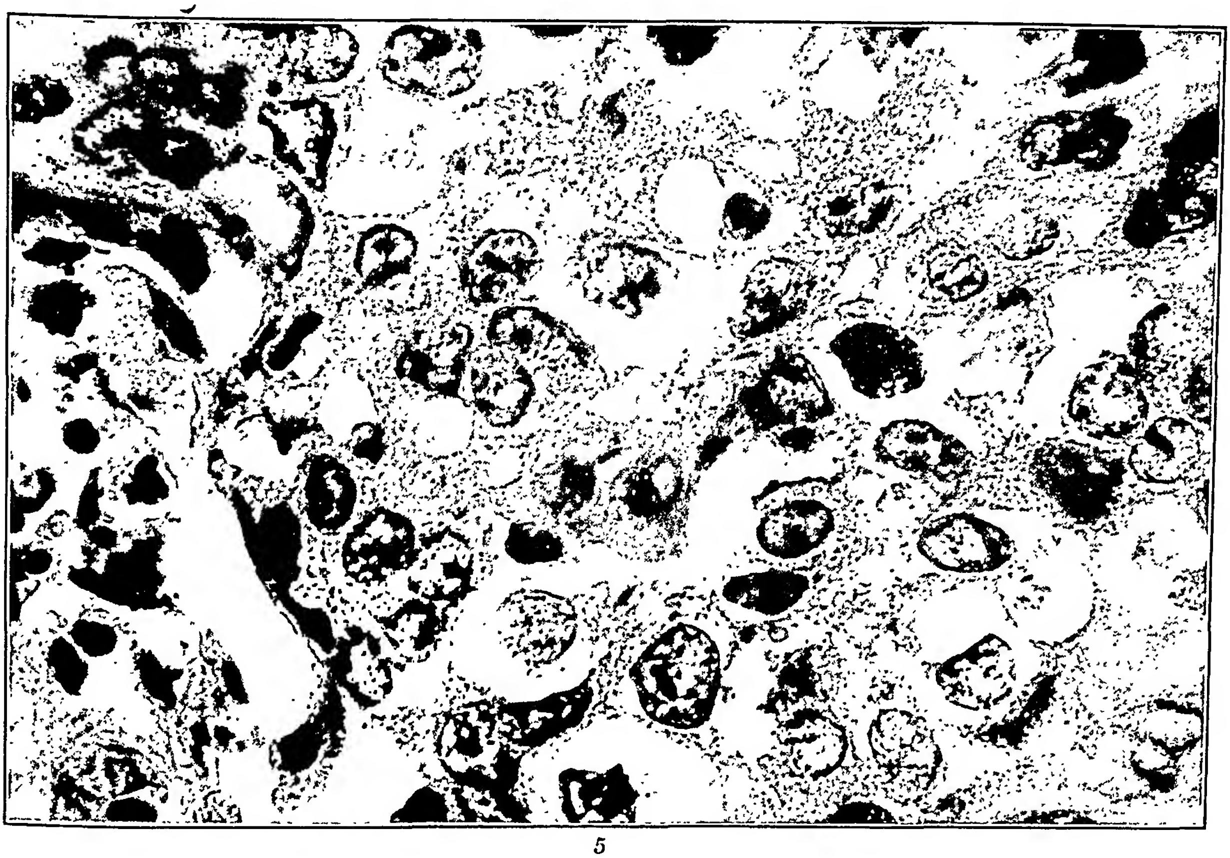

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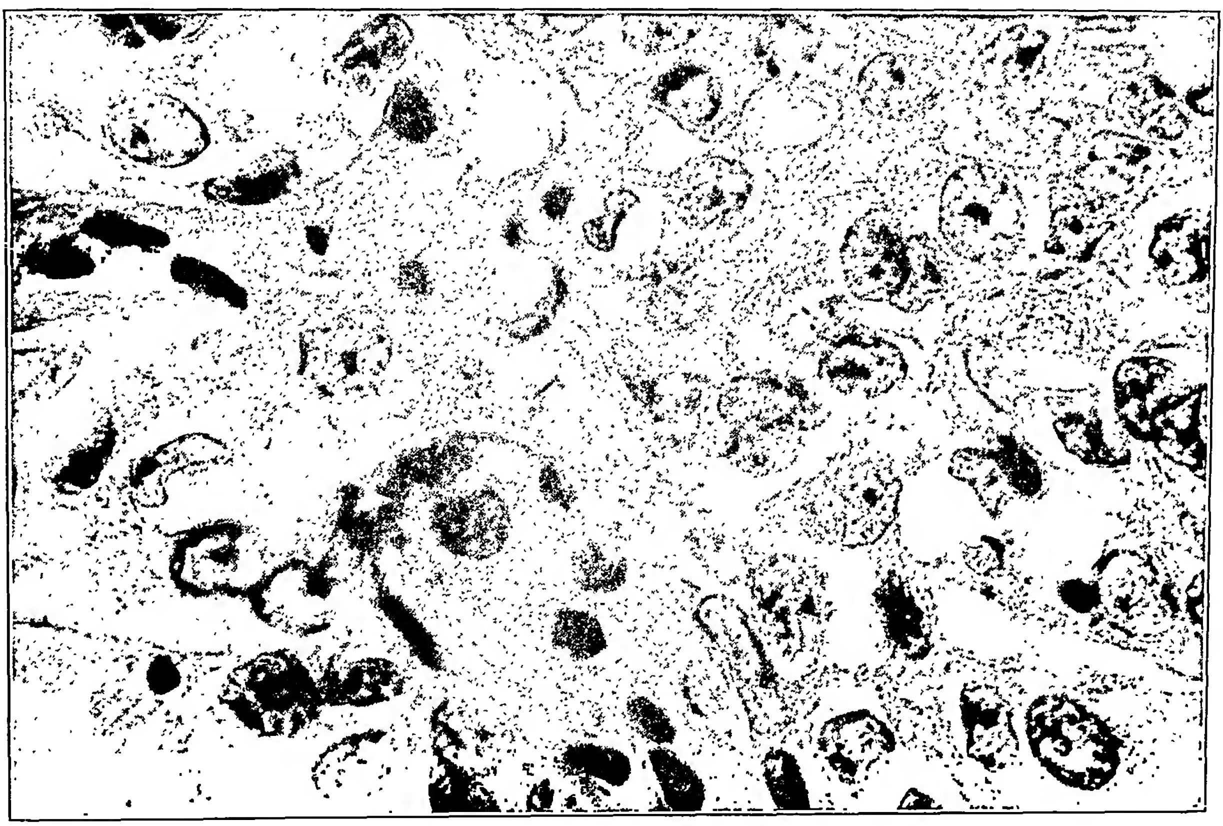
Foot

Malignant Thymoma

6



7

Foot Malignant Thymoma

THE FATE OF REACTING LEUCOCYTES IN THE TUBERCULIN AND REINFECTION REACTIONS *

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INTRODUCTION

In two previous studies [1,2] concerned with the tuberculin reaction and the reaction to tuberculous reinfection in testes of hypersensitive guinea-pigs, one of the authors noted a rather marked phagocytosis of reacting polymorphonuclear leucocytes by endothelial leucocytes. The question naturally arose as to whether or not these phagocytized polymorphonuclears were dead before they became engulfed. It is believed that solution of this question might have a certain theoretical value in interpreting the protective significance of the tuberculin and reinfection reactions.

Nearly all laboratory workers in tuberculosis believe that when an animal is tuberculin hypersensitive, there is a certain variable degree of immunity toward reinfection with the tubercle bacillus. It is likewise quite tacitly assumed that the two phenomena are causally related; that is to say, the more immediate inflammatory reaction to reinfection in the hypersensitive animal is responsible for the slower dissemination of bacilli, which is known to occur.[3] There are, however, certain quite apparent objections to this ready assumption. In the tuberculin and in the reinfection reaction the leucocytes forming the bulk of the exudate are polymorphonuclear; so far as is known, the polymorphonuclear leucocyte phagocytizes tubercle bacilli but does not destroy them; consequently the rôle played by these cells in the reaction is problematical. The mononuclear leucocyte is believed by some to destroy tubercle bacilli and Krause and Peters [4] observed in the reinfection reaction an earlier appearance of mononuclears than in non-sensitized control animals

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with primary infection. To them this observation suggests an explanation of the protective value of sensitization. Recently Cunningham, Sabin, Sugiyama and Kindwall [5] have denied that the monocyte destroys tubercle bacilli. We do not know the reason for the polymorphonuclear or for the mononuclear reaction in reinfection. Our own observations in the past have led us to favor its interpretation as a response to local tissue injury. Whether this is the sole explanation or whether some further mechanism comes into play, cannot be determined on the basis of present knowledge. It has seemed to us desirable to know if the leucocytes themselves share in the general sensitization of the tuberculous animal and the present study is concerned with this point.

Methods

One method of demonstrating sensitization is to show whether or not the supposedly sensitized cell undergoes necrosis when exposed to specific agents, in this case tuberculin, which to the cells of normal animals are innocuous. We first attempted to obtain a non-specific exudate in an animal sensitized by killed tubercle bacilli by the use of aleuronat and to incubate this exudate with varying amounts of old tuberculin, determining the death of the leucocytes by Nakayama's method.[6] The method is cumbersome, ill-suited to small amounts of material and uncertain; after a few trials it was abandoned. It was next decided to attempt the reaction *in vivo*, withdrawing the exudate from time to time and determining the condition of the cells by supravital staining with neutral red and Janus green following the technic of Sabin and her collaborators.[7, 8]

The important point in the supravital technic is to have a clean glass slide evenly covered with the vital dye. Glass slides are placed in a solution of concentrated sulphuric acid and potassium bichromate for three days. The slides are then washed in tap water for one day, rinsed thoroughly with distilled water and stored in 80 per cent alcohol. Saturated alcoholic stock solutions of Janus green and neutral red are prepared. A dilute solution of neutral red is then made by adding twenty drops of the stock solution to 5 cc. of absolute alcohol. Three drops of the saturated Janus green solution are added to 2 cc. of the dilute neutral red solution. This gives the dilution of the combined dye necessary for the differential

staining of the supravital technic. The glass slides are then removed from the 80 per cent alcohol by means of forceps, dried with a clean piece of gauze, flamed and then flooded with the combined dye which is quickly drained off so as to obtain an equal distribution on drying. It is essential that the fingers touch neither the slide nor the dye lest there be contamination with small amounts of grease. The stained slides are then stored in a slide box until they are used.

A series of guinea-pigs were sensitized by the injection of killed tubercle bacilli into the peritoneal cavity. Three injections, each 2.5 mg. dry weight of bacilli, sufficed. Ten days after the last treatment all of the animals reacted strongly to an intracutaneous inoculation of old tuberculin in 1:20 dilution. With one exception, the skin tests did not give necrosis, showing only marked erythema, induration and central blanching. Several days elapsed between the skin testing and the examination of the exudates. The latter were obtained as follows: two guinea-pigs, one sensitized and one control, each received 4 cc. of hormone broth containing 0.1 cc. of old tuberculin into the right pleural cavity; no immediate reaction resulted, save for a temporary series of hiccoughs. Twenty-four hours later the exudates were withdrawn, using glass tuberculin syringes previously subjected to the cleansing treatment described for slides covered with the stain. Syringes were warmed to body temperature before use. Needles were cleaned in alcohol followed by ether. A drop of the exudate was placed on a cover slip which was quickly inverted on the warm slide; the preparation was ringed with vaseline of high melting point and transferred to a microscope in a warm box maintained at a temperature of 37 to 38 C. Fluids from control and sensitized animals were placed on the same slide to avoid errors due to concentration of dye or other possible difficulties. This procedure was repeated at forty-eight hours and in some instances again at seventy-two hours.

At this point it becomes necessary to discuss the criteria for identifying the living, injured and dead cells observed in the supravital preparations of the exudate (Figs. 1 to 8). The polymorphonuclear leucocyte is characterized in the supravital preparation by its motility. These cells in their most active state show a streaming of the neutrophilic granules with the nucleus following behind. In the resting state these cells are round and show slight streaming and Brownian movement of the neutrophilic granules. The monocyte

shows motility, but to a less degree than the polymorphonuclear. In these cells there is a clear centrosphere in the cytoplasm occupying the indented portion of the nucleus surrounded by a rosette of small pink-staining bodies. Outside the rosette are the larger vacuoles staining intensely with neutral red. The mitochondria staining a deep bluish green with the Janus green are always on the periphery of the vacuoles. In the monocyte phagocytized material is always placed peripherally to the rosette of small bodies and the centrosphere is never obscured. The monocytes become round in the inactive stage. The clasmatocytes are large cells showing very little motility and a remarkable phagocytic ability. No centrosphere or rosette is visible and the mitochondria are not as prominent as in the monocyte. The phagocytized material is distributed throughout the cytoplasm. The material first phagocytized is placed close to the nucleus and the cell fills up with débris irregularly and according to no definite pattern. These cells exhibit vacuoles of different sizes staining in varying degree with neutral red. The lymphocytes are characterized by very slow ameboid movement, the nucleus preceding the cytoplasm. In the inactive state they are round and show very little cytoplasm. There are numerous rod-shaped or dot-like mitochondria and very often one or two small neutral red staining bodies localized in one part of the narrow rim of cytoplasm. In the supravital preparation, cell injury is denoted by rounding of the cells, together with cessation of the streaming motions of the granules. Then as the cell dies there is a gradual diffusion of the neutral red throughout the protoplasm of the cell together with a concentration of the granules, vacuoles, mitochondria and nuclear material in the center of the cell. The nucleus is deeply stained, whereas in the living cell it is unstained.

PROTOCOLS

GUINEA-PIG 244 (sensitized) received 4 cc. hormone broth with 0.1 cc. O. T. in right pleural cavity. Twenty-four hours later the exudate contained:

dead polymorphonuclears, 81 per cent; living, none;
dead lymphocytes, 1 per cent; living, none;
dead monocytes, 18 per cent; living, none.

No further examinations were made.

The corresponding control guinea-pig received identical treatment. The pleural fluid showed:

living polymorphonuclears, 26 per cent; dead, none;
living eosinophiles, 14 per cent; dead, none;
living monocytes, 57 per cent; dead, none;
living clasmatocytes, 3 per cent; dead, none.

No subsequent examination was made.

GUINEA-PIG 242 (sensitized) received broth and tuberculin as did the above. Twenty-four hours later the exudate consisted of:

dead polymorphonuclears, 92 per cent; living, none;
dead monocytes, 8 per cent; living, none.

At 72 hours:

living polymorphonuclears, 21 per cent; dead, none;
living basophiles, 1 per cent; dead, none;
living eosinophiles, 4 per cent; dead, none;
living small lymphocytes, 21 per cent; dead, none;
living monocytes, 40 per cent; dead, none;
living clasmatocytes, 13 per cent; dead, none;
living clasmatocytes, 8 per cent; dead, none.

The control animal showed at 24 hours:

living polymorphonuclears, 79 per cent; dead, none;
living eosinophiles, 4 per cent; dead, none;
living small lymphocytes, 1 per cent; dead, none;
living monocytes, 16 per cent; dead, none.

At 72 hours:

living polymorphonuclears, 29 per cent; dead, none;
living basophiles, 3 per cent; dead, none;
living eosinophiles, 1 per cent; dead, none;
living small lymphocytes, 5 per cent; dead, none;
living monocytes, 54 per cent; dead, none;
living clasmatocytes, 8 per cent; dead, none.

GUINEA-PIG 245 (sensitized) was treated as above. In 24 hours the exudate showed:

dead polymorphonuclears, 92 per cent; living, none;
dead small lymphocytes, 2 per cent; living, none;
dead monocytes, 6 per cent; living, none.

At 48 hours:

living polymorphonuclears, 17 per cent; dead, 14 per cent;
living lymphocytes, 12 per cent; dead, none;
living monocytes, 56 per cent; dead, none;
living clasmatocytes, 1 per cent; dead, none.

At 72 hours:

living polymorphonuclears, 5 per cent; dead, none;
living lymphocytes, 20 per cent; dead, none;
living monocytes, 61 per cent; dead, none;
living clasmatocytes, 14 per cent; dead, none.

The corresponding control gave at 24 hours:
living polymorphonuclears, 88 per cent; dead, none;
living eosinphiles, 3 per cent; dead, none;
living lymphocytes, 1 per cent; dead, none;
living mononuclears, 8 per cent; dead, none.

At 48 hours:
living polymorphonuclears, 65 per cent; dead, none;
living eosinophiles, 1 per cent; dead, none;
living lymphocytes, 1 per cent; dead, none;
living monocytes, 31 per cent; dead, none;
living clasmatocytes, 1 per cent; dead, none;
living serosal cells, 1 per cent; dead, none.

At 72 hours:
living polymorphonuclears, 28 per cent; dead, none;
living lymphocytes, 6 per cent; dead, none;
living monocytes, 60 per cent; dead, none;
living serosal cells, 6 per cent; dead, none.

GUINEA-PIG 241 (sensitized). Identical treatment was given as above. At 24 hours:
dead polymorphonuclears, 95 per cent; living, none;
dead lymphocytes, 5 per cent; living, none.

At 48 hours:
living polymorphonuclears, 33 per cent; dead, 48 per cent;
living monocytes, 18 per cent; dead, none;
living clasmatocytes, 1 per cent; dead, none.

At 72 hours:
living polymorphonuclears, 49 per cent; dead, none;
living eosinophiles, 9 per cent; dead, none;
living lymphocytes, 11 per cent; dead, none;
living monocytes, 28 per cent; dead, none;
living clasmatocytes, 3 per cent; dead, none.

The control guinea-pig showed at 24 hours:
living polymorphonuclears, 87 per cent; dead, none;
living monocytes, 12 per cent; dead, none;
living clasmatocytes, 1 per cent; dead, none.

At 48 hours:
living polymorphonuclears, 67 per cent; dead, 1 per cent;
living eosinophiles and basophiles, 1 per cent each; dead, none;
living monocytes, 27 per cent; dead, none;
living clasmatocytes, 3 per cent; dead, none.

At 72 hours:
living polymorphonuclears, 61 per cent; dead, none;
living eosinophiles, 3 per cent; dead, none;
living monocytes, 36 per cent; dead, none.

Guinea-pig 239 (sensitized). This animal, instead of receiving tuberculin, was given a suspension of killed tubercle bacilli made by adding 1 cc. of saline suspension of bacilli, containing 2.5 mg. per cc., to 2 cc. of hormone broth. At 24 hours the exudate contained:

living polymorphonuclears, 53 per cent; dead, 20 per cent;
living eosinophiles, 7 per cent; dead, none;
living monocytes, 20 per cent; dead, none.

At 48 hours:

dead polymorphonuclears, 89 per cent; living, none;
dead lymphocytes, 9 per cent; living, none;
dead monocytes, 1 per cent; living, none;
dead clasmatocytes, 1 per cent; living, none.

At 72 hours:

living polymorphonuclears, 38 per cent; dead, 2 per cent;
living eosinophiles, 4 per cent; dead, none;
living lymphocytes, 16 per cent; dead, none;
living monocytes, 30 per cent; dead, none;
living clasmatocytes, 10 per cent; dead, none.

The control under similar treatment showed at 24 hours:

living polymorphonuclears, 81 per cent; dead, none;
living eosinophiles, 4 per cent; dead, none;
living monocytes, 15 per cent; dead, none.

At 48 hours:

living polymorphonuclears, 55 per cent; dead, none;
living lymphocytes, 2 per cent; dead, none;
living monocytes, 37 per cent; dead, none;
living clasmatocytes, 6 per cent; dead, none.

At 72 hours no fluid could be obtained.

It is interesting to note the variation in phagocytosis occurring from day to day in both sensitized and control animals. At the end of twenty-four hours there were very few monocytes and practically no clasmatocytes present in the exudate and very little phagocytosis by these cells. After forty-eight hours the exudate showed numerous phagocytic cells, chiefly monocytes, containing débris and whole polymorphonuclears; after seventy-two hours the monocytes and clasmatocytes were again increased in numbers and in phagocytic activity. It was not uncommon to see a clasmatocyte containing from three to seven polymorphonuclears and monocytes full of cellular débris. Phagocytosis was more marked always in the later stages of the reaction in the sensitized animal than in the control.

Discussion

The results derived from this series of experiments are difficult to interpret in immunologic terms. It is certain that evidence points to the fact that in the tuberculin and reinfection reactions the reacting cells are sensitized and in early stages of the reaction undergo necrosis. With a diminution of the intensity of the reaction due possibly to the absorption of the exciting antigen, barely possibly to a desensitization, new polymorphonuclears appear as a reaction to tissue débris and with them come large numbers of mononuclear phagocytes. The whole phenomenon suggests that the leucocytes appearing in the tuberculin reaction do not have the protective significance commonly ascribed to them. To determine this point a huge series of animals would be necessary, with varying reinfecting doses of living organisms and correlation of spread of disease with the results of studying the behaviour of cells in the immediate reaction. One other interpretation is possible: the observed diminished rate of spread of reinfecting organisms in a hypersensitive animal may be due to necrosis and disintegration of the cell carrying them, this necrosis occurring near the source of inoculation rather than at a distant focus. No positive conclusion as to the immunologic significance of facts so far observed is justified.

Conclusions

In the tuberculin and reinfection reactions in tuberculin hypersensitive animals, the reacting inflammatory cells appear to be themselves sensitized. Treated with appropriate doses of antigen, they undergo early death.

These first cells are gradually replaced by others probably reacting largely to the presence of necrotic tissue. The newly emigrating cells are viable elements.

Further study is required to determine the immunologic significance of these facts.

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DESCRIPTION OF PLATE

Plate 15

Fig. 1. Dead polymorphonuclear neutrophile; nucleus deeply stained, cytoplasm diffusely stained with the granules clumped about the nucleus leaving a peripheral clear zone.

Fig. 2. Dead monocyte; a narrow peripheral clear zone within which is diffusely stained cytoplasm and many neutral red bodies. At the upper right a dead phagocytized polymorphonuclear; beneath this the faint, diffusely stained nucleus of the monocyte.

Fig. 3. Motile, active polymorphonuclear neutrophile; nucleus unstained; granules occasionally stained; several pseudopods.

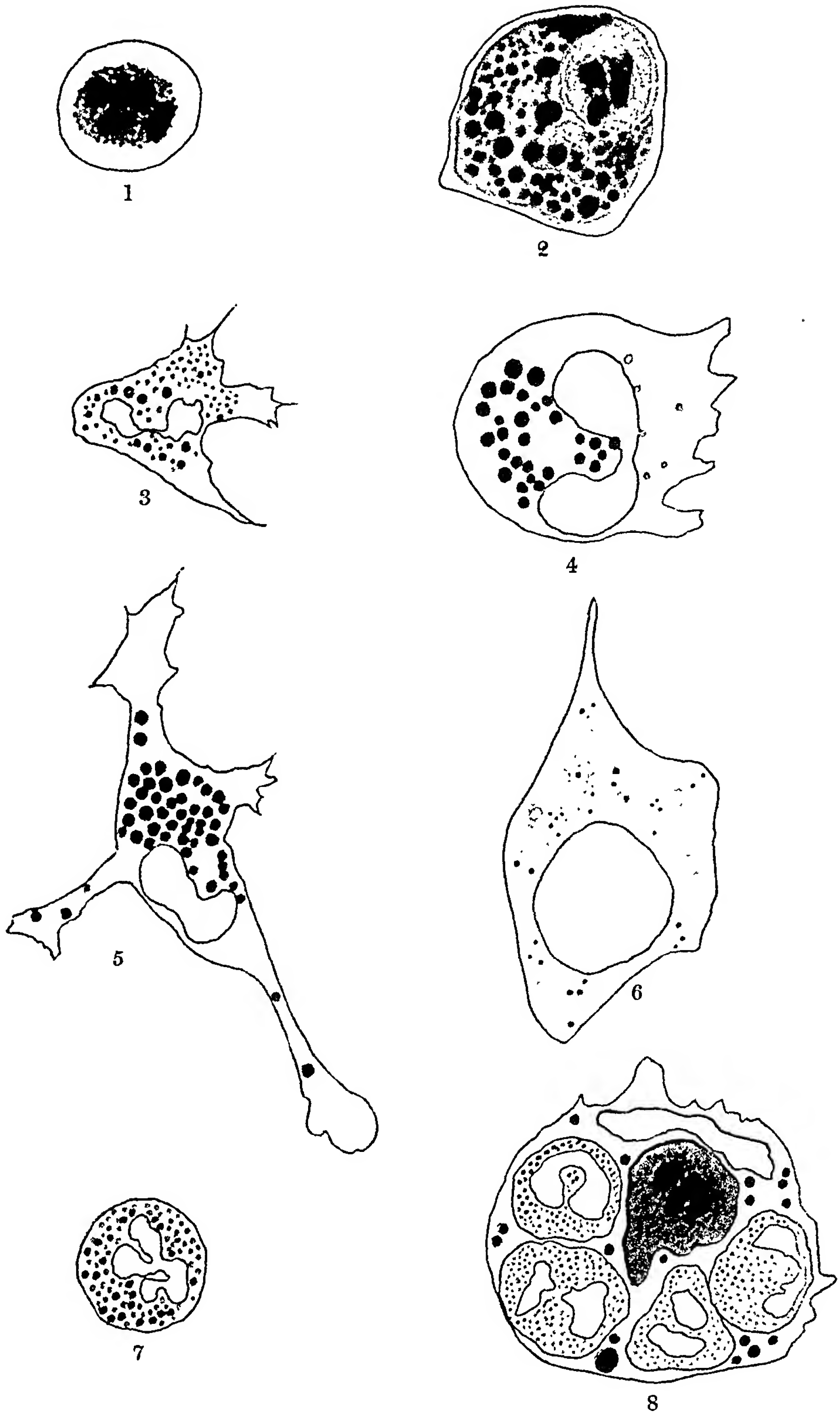
Fig. 4. Resting monocyte; crescentic unstained nucleus; neutral red staining material occupying the cytoplasm adjacent to the hollow crescentic portion of nucleus; a few small pseudopods.

Fig. 5. Very active monocyte; numerous long pseudopods.

Fig. 6. Living serosal cell; nucleus unstained; no neutral red staining material.

Fig. 7. Resting polymorphonuclear neutrophile. The cell is rounded; granules fill out the cytoplasm; nucleus unstained.

Fig. 8. Living clasmatocyte. Flattened eccentric nucleus, unstained; neutral red staining material scattered sparsely throughout cytoplasm; five phagocytized polymorphonuclears, four of which are living and one (stained) dead.



Stewart, Long and Bradley   Fate of Leucocytes in Tuberculin Reaction

THE PERSISTENCE OF THE GLOMERULAR CIRCULATION FOLLOWING OCCLUSION OF THE RENAL VEIN OF ONE KIDNEY IN THE CAT*

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Only the immediate pathologic changes consequent to thrombosis of the renal vein in man are well known, but possibly certain lesions found in the kidney are the late results of previous venous occlusions which have been survived, and the changes are not recognized as lesions resulting from this occurrence. With a view of determining experimentally whether any characteristic changes followed venous obstruction of the kidney, the left renal vein in a series of cats was cut between ligatures, and the animals killed at varying intervals of time thereafter. The resulting changes seemed to furnish data on the finer circulation of the kidney not furnished by other experimental methods.

In brief, the distinctive feature of the picture following venous obstruction is the preservation of glomeruli in contrast to the widespread destruction of tubules. The early changes consisted of congestion and edema followed by degeneration and necrosis of convoluted tubules, most marked in the peripheral zone of the cortex. Beneath this peripheral zone is another zone of less marked degeneration of tubules where glomeruli, though showing changes in the capsular epithelium, were relatively undamaged. At a later period the glomeruli presented a healthy appearance, while there was complete destruction of tubules. At the end of the second month there was an extremely atrophied organ, reduced to about one-twentieth the volume of the opposite kidney, and consisting almost wholly of intact, closely approximated glomeruli.

Injections of Berlin blue through the renal artery of these atrophied kidneys showed a free circulation through the glomeruli. The path of this circulation could be traced through the kidney in microscopic sections of the injected organ, from the main renal artery and its larger branches, through afferent arterioles to the glomerulus; and

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thence through postglomerular vessels to extrarenal anastomosing channels. That the anastomosing circulation occurred through already existing channels was indicated by the uniformity of the results and the similarity of the collateral circulation traced in the case of normal and atrophied kidneys. It was clearly established that this collateral circulation involved only the glomeruli and excluded the tubules, both convoluted and collecting. In other words, the vessels to the tubules alone are terminal vessels, and this is responsible for their complete destruction following vascular stasis. In contrast to the blood supply to the tubules, the circulation through the glomeruli was found to be an extremely free one, as the injection with Berlin blue was accomplished more readily and with less pressure than in the case of the normal kidneys. When adhesions occurred between the omentum and the injured kidney, injections of blue showed that no anastomosing channels were established through these points of adhesions.

There is then a certain independence of the vascular supply of the glomeruli from that of the tubules and this can be conceived of as having some bearing upon the functional independence of these two elements.

Experimental Procedure

Young adult cats were anesthetized and the left renal vein cut between ligatures. In several instances the spermatic or ovarian veins were also sectioned. At varying intervals of time up to four months, the animals were killed. In all cases, the vessels were carefully dissected after death to study the collateral circulation and to prove that the renal artery was patent and that the renal vein had been cut proximal to its tributaries.

In several instances while the kidneys lay *in situ*, the renal arteries were injected with a 2 per cent solution of Berlin blue and the course of the injected vessels studied in thick microscopic sections with a binocular microscope.

The operation was performed on fourteen cats and one dog. Typical experiments have been selected from the series and are described below in the order of the time interval following ligation.

Experiment 1. Cat 17. Thirty-two hours after ligation.

Grossly, the kidney is swollen, tense and purple. Blood has extravasated into the retroperitoneal tissues. The capsule strips

readily, disclosing mottled petechial hemorrhages on the surface of the kidney. On section, the medulla is dark purple and is clearly demarcated from the pale opaque swollen cortex in which all markings are obscured.

Microscopically, there is complete necrosis of certain segments of the tubules, while other segments are well preserved and the glomeruli remain intact. The distribution of necrosis is so regular that a definite pattern is formed. The periphery of the cortex immediately beneath the capsule contains nothing but tubules, cells of which have lost all nuclei; the cytoplasm stains deeply with eosin and fills up the entire lumen. The basement membrane remains intact, and sharply defines the outer border of each degenerated tubule. From this outer necrotic zone the loops of Henle extend inward as wedge-shaped areas of degenerated tubules. Collecting tubules traversing the pyramid are similarly necrotic. In the zone between the wedge-shaped areas of necrotic tubules, relatively intact, proximal convoluted tubules surround each glomerulus. Their cuboidal cells contain clearly stained nuclei and have only slightly vacuolated cytoplasm. The glomeruli are without lesions. Their capillaries appear patent and their capsules are not thickened. The subcapsular space is clear. All glomeruli appear much the same. There is none showing any stage of fibrosis or atrophy. The medulla contains only necrotic tubules in which the basement membrane remains intact, and many engorged capillaries. There is no cellular reaction around the necrotic tubules. The main vein is occluded by thrombosis.

Experiment 2. Cat 19. Eight days after ligation.

Grossly, the left kidney is of about the same size as the right. The capsule is slightly adherent, due to organization of mottled areas of subcapsular hemorrhage. On section the cortex presents a peripheral rim of bright yellow sharply defined by a narrow hemorrhagic zone from the dull grayish yellow appearance of the inner portion of the cortex. A hemorrhagic zone separates the inner border of the cortex from the medulla.

Microscopically, necrosis of tubules has occurred according to a definite pattern. In the periphery of the cortex there is complete necrosis of tubules and of the few glomeruli that are in this area (Fig. 1). This zone is clearly defined by its intense staining of the necrotic protoplasm with eosin. All nuclei have disappeared from

the epithelial cells. The outlines of tubules, however, are indicated by the intact basement membrane and by the fact that fibroblasts are proliferating in the interstices between preexisting tubules. This proliferation of fibroblasts is from the surface of the kidney and the cells are penetrating inward. The necrotic tubules extend from the periphery downward, forming triangular areas with the apices pointing inward and reaching the medulla, and representing the loops of Henle and collecting tubules.

In the deeper zone of the cortex between the descending columns of necrotic tubules, are tubules that show some regeneration of the epithelial cells. Their cellular appearance contrasts markedly with the necrotic tubules. Their nuclei are large and closely placed, the cells being heaped upon the basement membrane in an irregular manner without definite alignment. Other tubules in this zone contain casts of hyaline material.

In the medulla the tubules are necrotic, only the basement membrane indicating preëxisting tubules. Capillaries here are patent and engorged. Near the pelvis and near the cortico-medullary junction fibroblasts are proliferating.

Glomeruli throughout, except in the peripheral zone, are well preserved. Their capillaries contain red cells. The subcapsular space is of usual size and no thickening of the capsule has occurred. The glomeruli are closely placed due to destruction of intervening tubules.

EXPERIMENT 3. Cat 30. Seventeen days after ligation.

In this case part of the collateral circulation had been cut off by ligating the ovarian vein as well as the renal vein.

Grossly, the omentum, spleen and pancreas are adherent to the left kidney. There are distended vessels in the lateral abdominal wall near the kidney. Blood has extravasated retroperitoneally. The left kidney is atrophied to about one-half the size of the right.

While the kidneys lay still *in situ*, the renal arteries of both sides were injected with 2 per cent Berlin blue. The solution passed into the left kidney more readily than into the normal one. The dye quickly filled the collateral circulation consisting chiefly of branches to the adrenal, to the lateral abdominal wall and lumbar region. The same collateral channels were injected on the normal side by injecting the right renal artery. No vessels were stained in the adhesions formed between omentum, pancreas and kidney.

The capsule of the left kidney can not be stripped away. On section, the deeper portion of the cortex is stippled by dark blue dots representing injected glomeruli which are more closely placed than in the normal kidney. There is a pale peripheral zone of the cortex which contains little blue pigment, while in the healthy kidney the pigment reaches the outermost portion of the cortex. A few blue strands traverse the pale medulla and reach the cortex and surface of the kidney. The lining of the pelvis stains a deep blue.

Microscopically, old hemorrhage beyond the capsule is in the process of organization and fibroblasts extend from without into the renal tissue. The cortex shows a peripheral zone of necrosis where tubules are defined only as masses of hyalinized protoplasm bordered by basement membrane and separated by fibroblasts.

Beneath this outer zone, the cortex contains tubules in various stages of degeneration and atrophy. Some tubules show regenerating cells which are atypical in arrangement, the cells being heaped up without definite alignment. The nuclei stain well and the cells are so numerous that the nuclei appear closely placed. These cells practically fill the lumina. The impression given is that the cells have regenerated due to connection with the glomerular circulation but that they are relatively undifferentiated in character and non-functioning. In some tubules the lumen is dilated and contains granular material or hyaline casts. Among these atypical tubules the glomerular tufts stand out prominently. Their capillaries are well filled with deep blue pigment. The capsules are not thickened and the subcapsular space is of usual size and empty.

In the medulla, the central portion is completely necrotic, only shadows of tubules remaining, while the pelvic border and cortico-medullary junction show proliferating fibroblasts and capillaries.

Throughout the sections, vessels filled with blue pigment are seen: (1) large arterial branches at the hilum, (2) arteries in cross section at the cortico-medullary junction, (3) afferent arterioles leading to the glomeruli, (4) longitudinal sections of postglomerular vessels running through all zones of the cortex, including the necrotic peripheral zone, and reaching the surface, (5) capillaries in the perirenal area of organizing hemorrhage and (6) capillaries in the medulla just beneath the pelvic lining. Thus the circulation can be traced from the artery at the hilum through perforating vessels which bear glomeruli, to the perirenal vessels.

In the normal right kidney the capillaries injected with blue are more numerous and are seen to pass to healthy tubules as well as to glomeruli. The injected glomeruli are identical in appearance to those of the left kidney.

Experiment 4. Cat 31. Five weeks after ligation.

The left ovarian vein had been ligated, as well as the renal vein. Adhesions have formed between the omentum and colon and the left kidney. While the kidneys lay *in situ*, the renal arteries were injected with Berlin blue. The collateral circulation thus injected was similar to that previously described. The adherent omentum and intestine were not stained by this injection, and consequently played no part in the collateral circulation.

There is some hemorrhage in the retroperitoneal tissues. The capsule is so adherent that it is impossible to strip it from the kidney. The kidney is smaller than the right. On section, the medulla is unstained except near the pelvis and at the cortico-medullary junction. The peripheral layer of the cortex is bright yellow, necrotic and contains no blue. The inner portion of the cortex is stippled by blue points representing injected glomeruli. In the normal kidney the medulla is deeply stained with blue and the entire cortex contains blue dots and striations.

Microscopically, the peripheral portion of the cortex contains no healthy tubules. A growth of fibroblasts extends inward from the capsule and penetrates in strands between the remains of tubules. Only the basement membranes of tubules remain and appear as small cystic spaces riddling the growth of connective tissue. In this zone no glomeruli are present. Large capillaries filled with blue extend through this zone to the surface.

The cortex beneath this outer zone contains healthy glomeruli well filled with blue (Fig. 2). An occasional glomerulus is atrophied. Tubules in this area are largely atrophied or show lumina filled with hyaline casts. The medulla is completely necrotic and contains no vessels filled with blue, except the portion immediately contiguous to the pelvis and to the cortex. The capillary circulation is quite similar to that previously described.

Experiment 5. Cat 20. Two months after ligation.

Grossly, the left kidney is reduced to an extremely small organ, measuring 1.8 × 1.0 × 0.6 cm., and weighing 2.1 gm., while the

right kidney measures 4.5 × 3.2 × 2.5 cm., and weighs 25.1 gm. The capsule strips readily. On section, the tissue is an orange-yellow color. The cortex is very narrow. The renal artery is patent.

Microscopically, a sharply defined peripheral zone of the cortex contains atrophied or hyalinized tubules and a diffuse growth of fibroblasts. Immediately beneath this zone, the cortex consists of many glomeruli, closely approximated because of atrophy of tubules (Fig. 3). A few extremely atypical tubules survive. The glomeruli appear patent, and the subcapsular space is of usual size. In the medulla, empty spaces apparently represent atrophied tubules. These are supported by connective tissue and capillaries.

Experiment 6. Cat 33. Two months after ligating renal vein.

The renal arteries of both sides were injected with Berlin blue while the organs lay *in situ*. The usual collateral circulation was thereby demonstrated. The left kidney is enormously atrophied measuring 1.5 × 1.3 × 1.0 cm., while the unligated right kidney measures 4.8 × 3.5 × 2.5 cm. The atrophied kidney shows only a narrow rim of uninjected cortex. The medulla is pale.

Microscopically, all tubules have atrophied or have disappeared, with only the supporting connective tissue remaining. Many glomeruli have likewise been destroyed, but those which persist have been permeable to the injected Berlin blue which is seen filling their capillaries. The glomeruli are closely packed due to atrophy of intervening tubules. Their capsules are thickened and the subcapsular space is enlarged.

Experiment 7. Dog 5. Three months after ligation.

Grossly, the left kidney is reduced to a minute organ measuring 2.8 × 1.9 × 1.2 cm., while the right measures 6.8 × 3.5 × 3.3 cm. The capsule strips readily. On section the tissue is of an orange-yellow color. The medulla is reduced to a narrow yellow zone. The renal artery is patent.

Microscopically, there is a clearly defined peripheral zone of the cortex in which tubules are completely atrophied and replaced by connective tissue, but in which the capillaries are patent. The entire cortex below this is composed of glomeruli, closely approximated because of atrophy of intervening tubules (Fig. 4). Some atypical tubules persist. Everywhere the capillaries are apparently patent.

The medulla consists of a diffuse growth of connective tissue and atrophied collecting tubules indicated chiefly by basement membrane.

In the case of three of the cats, in which the ovarian or spermatic veins had been ligated in addition to the renal vein, no collateral circulation was functioning and there was consequent complete necrosis of all elements. In other animals in which the ovarian vein had been ligated, as well as the renal, the changes were the same as when the renal vein alone had been ligated.

Discussion

In reviewing the literature, it was found that the changes within the kidney resulting from experimental venous occlusion had been described in 1876 by Buchwald and Litten [1] who attempted in this manner to reproduce the contracted kidney in rabbits and dogs. They describe especially the early stages of congestion and edema followed by destruction of tubules. They state that in contrast to the universal destruction of tubules, the preservation of the glomeruli is striking. The organ decreased markedly in size and the collateral circulation was abundant.

The circulation of the kidney has been studied usually with the object of determining the arterial distribution under normal and pathologic conditions by various injection and corrosion methods and by stereoscopic roentgenograms after injection of opaque solutions. These solutions do not usually pass beyond the glomerulus, so they give no information in regard to the postglomerular capillary circulation. The present study is not concerned with this grosser circulation but with the finer distribution of vessels. Recently Lee-Brown,[2,3] by injecting the renal arteries with Berlin blue solution and examining thick sections of the tissue with the binocular microscope, has elucidated many details in this circulation. By this method of using a diffusible pigmented fluid, he traced the path of capillaries which formed a postglomerular anastomosis. Further evidence of such an anastomosis was given by the fact that when the vein of an excised kidney was ligated and Berlin blue injected into one main division of the renal artery, there was a return flow of dye through the other main division of the renal artery. If barium sulphate was injected, no such return flow occurred, because the

particles of barium were caught in the glomerular tuft. This he considers gives further evidence that the anastomosis is a postglomerular one. Microscopically, he locates this anastomosis in the efferent plexus of the cortex and in the medulla, chiefly at the apex of the pyramids. The results of my experiments seem to give further evidence of a postglomerular anastomosis, but one which connects the glomeruli with the extrarenal collateral channels and which permits a circulation to the glomeruli independent of that of the tubules.

The recent experiments of Belt and Joelson [4] in injecting human and dog kidneys deal especially with possible arterial anastomoses after arterial obstruction. They show the constancy of perforating arteries which penetrate through the entire kidney, reach the perirenal tissue and take their course through perirenal fat or form extrarenal anastomoses with branches of the main renal artery or with branches of the renal artery which supply the pelvis and ureter. My experiments would indicate that in the cat after venous occlusion the circulation is effected through such vessels traversing the kidney and penetrating into the perirenal tissue. They would also show that most glomeruli are in free communication with such vessels, since the persistence of glomeruli after venous occlusion indicates that glomeruli commonly have such a close relationship to vessels of this type.

The extrarenal collateral circulation was found by Lindeman [5] and by Barney [6] in experimental hydronephrosis of dogs to consist chiefly of connections with the adrenal, lumbar and spermatic or ovarian vessels. Tuffier and Lejars [7] found in dissecting human cadavers that there were five main groups of anastomosing venous channels, namely, capsular-renal, capsular-mesenteric, capsular-suprarenal, capsular-spermatic and capsular-lumbar. The details of these extrarenal anastomoses are not immediately relevant to my experiments. The injection of the ligated cat kidneys make it evident that some such anastomosis exists, and that the circulation after the obliteration of the renal vein is maintained through already existing channels rather than through newly formed ones. In the present paper I am primarily concerned with the intrarenal circulation which communicates with these extrarenal anastomosing channels, and it has been shown how inadequate this circulation is for the tubules. For the maintenance of the glomeruli, however, it appears to be more than adequate, for when part of the collateral circulation was

cut off by ligating in addition the ovarian or spermatic vessels, the glomerular circulation was still maintained, and the gross and microscopic changes were the same as when the renal vein alone was cut.

How far these results in cats are comparable to the effect of venous occlusion in man is impossible to say, but there is a striking similarity of the gross picture of these experimental kidneys with that of cases of so-called "symmetrical necrosis of the kidney" reported in the literature and associated with pregnancy or occurring in the new-born (Klotz,[8] Bradford and Lawrence,[9] Glynn and Briggs,[10] Torrens,[11] Griffith and Herringham,[12] Jardine and Teacher,[13] Bamforth[14] and others). The gross appearance is complete necrosis of the peripheral portion of the entire cortex with a clearly demarcated hemorrhagic zone in the deeper portion of the cortex. The etiology of the condition is much disputed. Usually it has been attributed to endarteritis, but the evidence is unsatisfactory that this is the causative factor. Torrens attributes the condition to venous thrombosis, a view which Glynn and Briggs challenge, because, they state emphatically, there is no clinical or experimental evidence that occlusion of the renal vein will cause such cortical necrosis. The late microscopic changes cannot be compared because the human lesion is bilateral and rapidly fatal, but Bamforth described in his case degeneration of the tubules while glomeruli appeared normal, and thrombi were seen in many veins while arteries were free.

Summary

1. Ligation and section of the renal vein of one kidney in cats result in congestion and edema followed by a clearly defined zone of necrosis in the periphery of the cortex beneath which there is a zone where tubules are degenerating and glomeruli remain intact.

2. In the later stages, necrosis of tubules is widespread, while many glomeruli persist. The extensive destruction of tubules results in extreme atrophy of the organ which is made up almost wholly of closely approximated glomeruli and capillaries.

3. Injection of the capillary circulation with Berlin blue demonstrates the course of the blood from the main renal artery through afferent glomerular arterioles, then through postglomerular capillaries to the extrarenal anastomosing channels.

4. The persistence of glomeruli with destruction of tubules indicates that there is a certain independence of the glomerular circulation from that of the tubules.

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DESCRIPTION OF PLATES

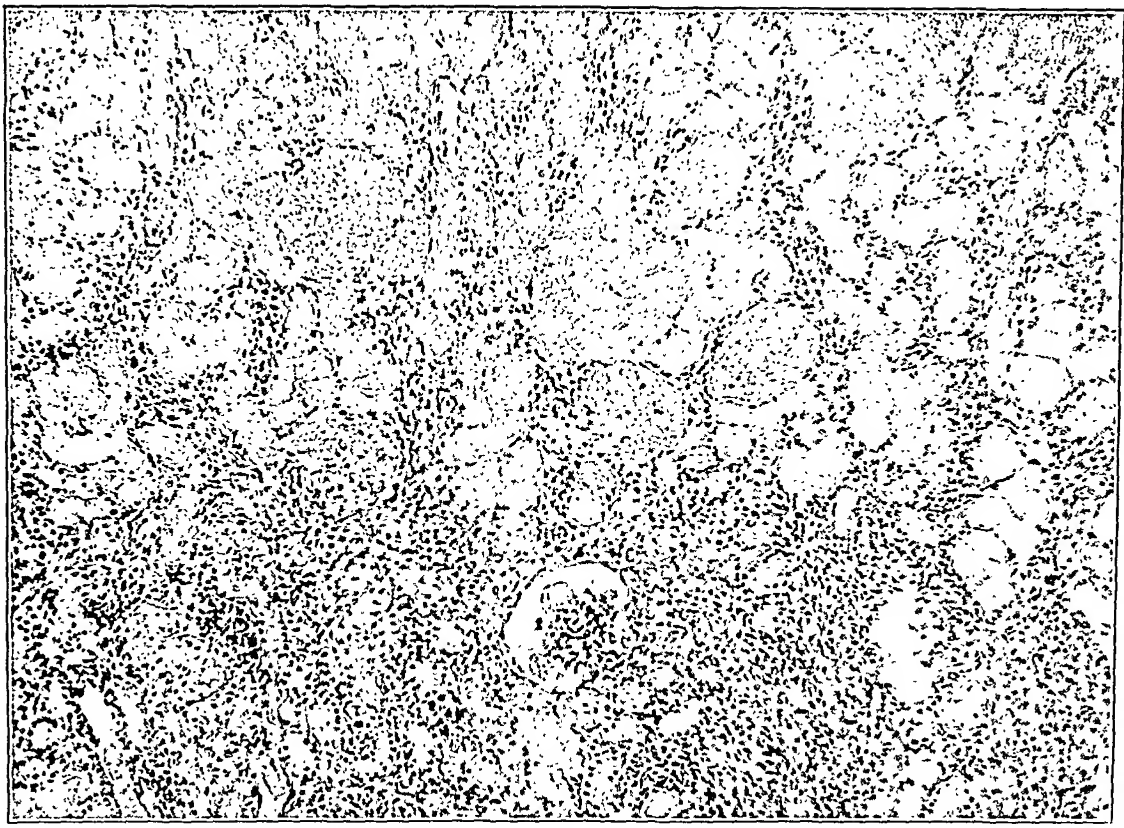
Plates 16-17

Fig. 1. Kidney of cat, eight days after transecting the renal vein, showing a sharply defined outer zone of complete necrosis, beneath which there is a zone of degenerating tubules. X 100.

Fig. 2. Kidney of cat, five weeks after transecting the renal vein. High power photomicrograph showing that the persisting glomerular tufts are patent to Berlin blue injection fluid. X 500.

Fig. 3. Kidney of cat, two months after transection of the renal vein. Glomeruli have persisted while tubules have atrophied or have completely disappeared. X 250.

Fig. 4. Kidney of dog, three months after transection of the renal vein. The kidney consists entirely of glomeruli and supporting connective tissue, while tubules have completely disappeared. X 50.



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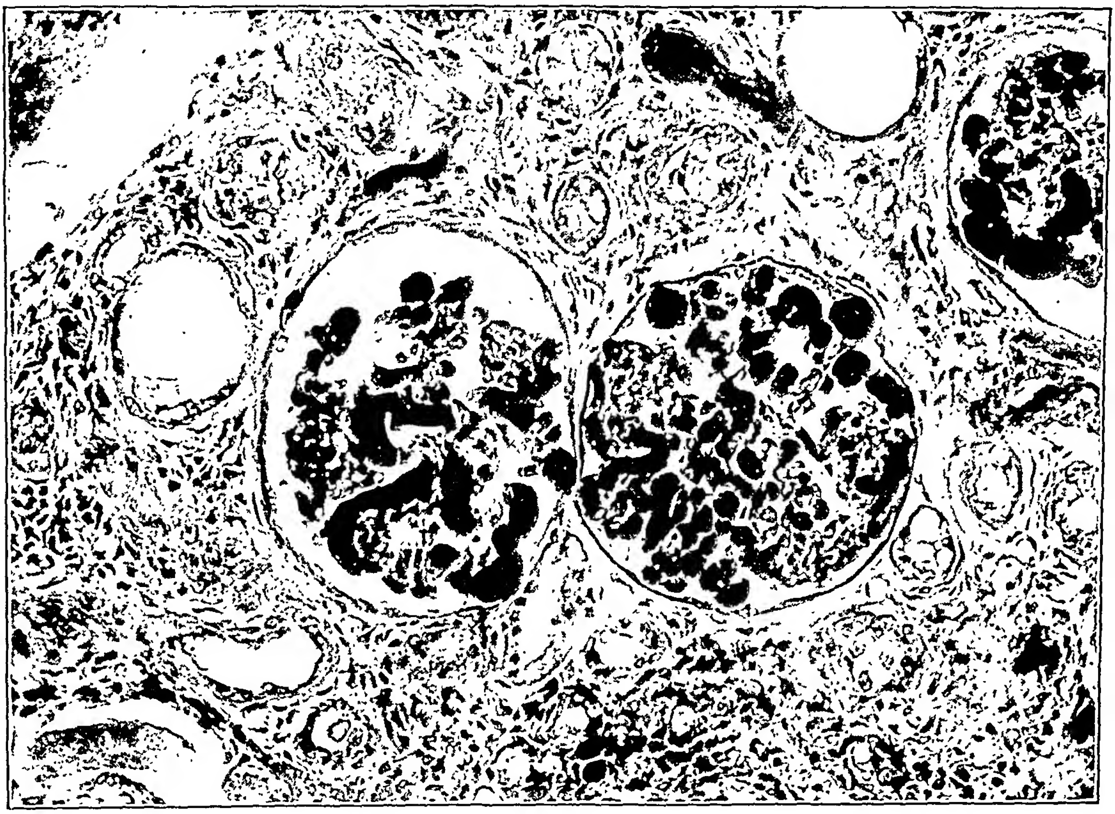

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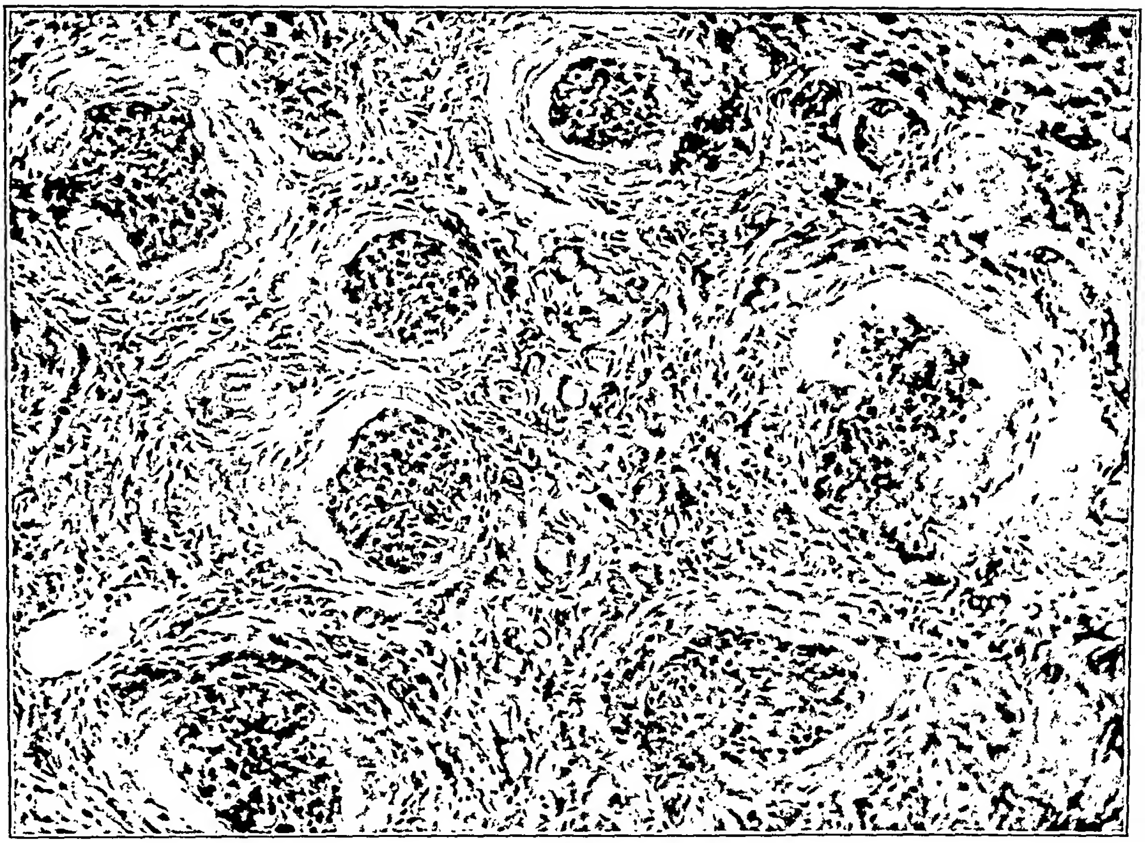

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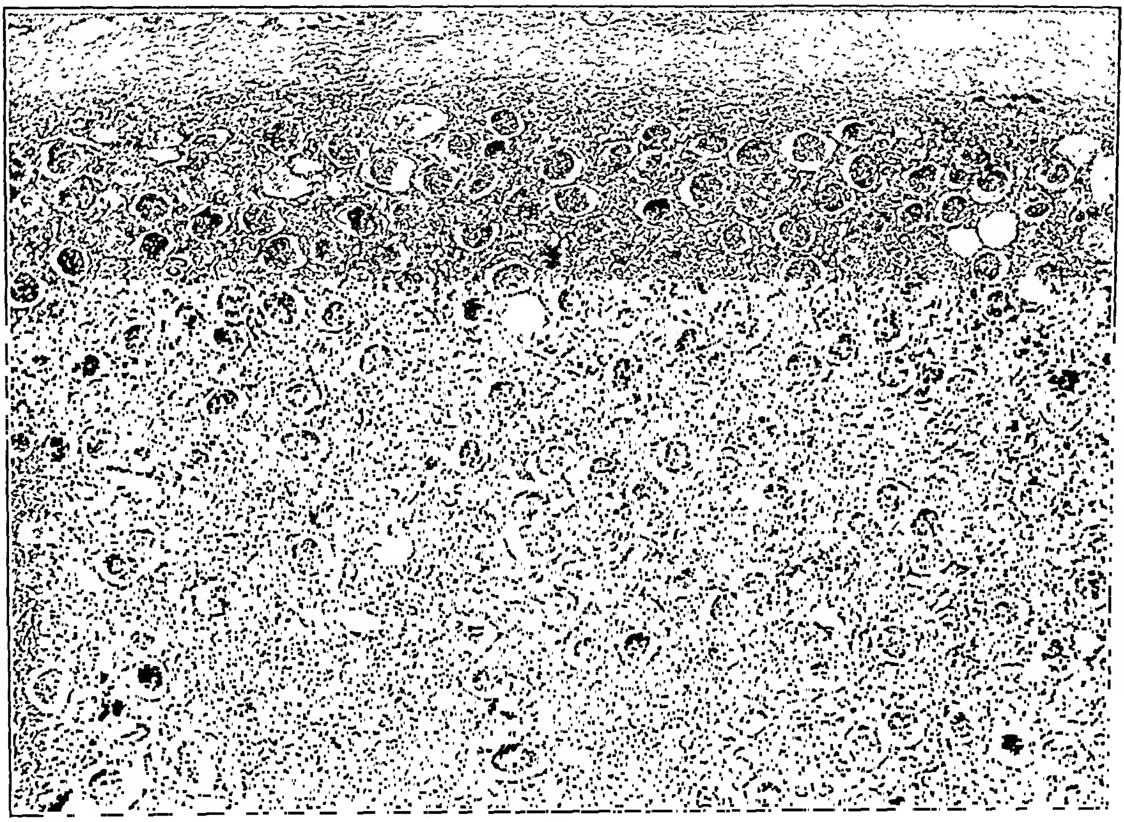

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Zeckwer The Glomerular Circulation

LIPOID-CONTAINING CELLS IN THE SPLEEN IN DIABETES WITH LIPEMIA *

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In the average spleen removed at necropsy there is little evidence, either gross or microscopic, of lipoid content. However Poscharisky[1] demonstrated fat to be present in spleens removed from cases of many different diseases and to be particularly abundant in those spleens containing amyloid and hyaline masses. This fat is present in small droplets and gives the characteristic staining reactions for neutral fat. He further states that in children the fat is chiefly within the "epithelioid" cells of lymph nodules of the spleen, while in adults it is found in the pulp, trabeculae, capsule and vessel walls.

Kusunoki[2] added further proof of the frequent occurrence of lipoid-containing cells in the spleen, and showed that most of the lipoid was contained in cells of the reticulum. The lipoid gave the following staining reactions: red with Sudan III, black with osmic acid and blue or blue-violet with Nile blue sulfate. Kusunoki also found the number and prominence of the lipoid-containing cells in the spleen to run parallel with the lipoid content of the blood.

Aside from this fairly common finding of lipoid-containing cells in the spleen, there are a few cases recorded in which very marked hyperplasia of these cells occurs, and the lipoid present does not as a rule give the staining reactions usually encountered. These cases are commonly associated with diabetes with lipemia.

The first instance of this striking change in the spleen was mentioned by Coats.[3] The patient suffering from diabetes with lipemia had died in coma. Large lipoid-containing cells made up most of the splenic tissue. Nine other cases have since been reported. The findings in eight of these † are summarized and compared in Table I with those of our three cases.

* Received for publication December 20, 1925.

† One case has been reported by Eppinger, according to Oppenheimer and Fishberg.[4] His publication is not accessible to us.

TABLE I

Comparison of Staining Reactions of Various Fats Found in the Spleen

Case	Type of Lipoid	Sudan III	Scarlet Red	Nile Blue Sulfate	Osmic Acid	Smith-Dietrich	Anisotropic Crystals
I Case No. 1305	1 2	Red Yellow	Red Faint	Blue Faint rose	Black Faint	+ +	few few
II Case No. 1794		Yellow	Faint	Faint rose	Faint brown	+	few
III Case No. 05–172		Orange	Red				
IV Schultze[5]		Faint	Faint	Faint rose	–	+	–
V Lutz[6]	1 2 3	Faint Red Red		Faint rose Blue Bright rose	= – Black		few
VI Lutz[6]	1 2	Red Yellow-red		Rose Violet	Black Black		
VII Marchand[7]		Yellow			Faint		
VIII Williams and Dresbach[8]		+		+			+
IX Smith[9]	1 2	Red Faint	Red Faint	Blue Faint rose	Black Faint	+ Gray reticulation	– –
X Fahr and Stamm[10]		–		–			+
XI Oppenheimer and Fishberg[4]		–				–	

NOTE: In some of the cases there are various types of lipoid present.

Of the three instances of this rare lesion reported in this paper, two were encountered in studying pathologic material placed at our disposal by Dr. Elliott P. Joslin. The third was brought to our at-

tention by Dr. F. B. Mallory. This last case has not been studied as carefully as we should wish since the necropsy was performed twenty years ago and the material other than the slides has been lost. However, we are particularly fortunate in that our first two cases were under careful observation for five and eight years respectively.

Case Histories

Case I. No. 1305. White male. Onset of diabetes at 10 years and 6 months of age. Maximum body weight (clothed) 75 lbs. (12 per cent above normal). The illness lasted five and one-half years. The

Table II

Blood Changes in Case I, No. 1305

Date	Blood Sugar gm. per 100 cc.	Blood Fat* per cent	
May 22, 1917	0.09	..	
Nov. 17, 1919	0.21	..	
Dec. 1, 1919	..	0.51	
June 3, 1921	0.25	1.00	
Jan. 3, 1922	0.24	1.68	
Feb. 13, 1922	0.22	1.72	
Aug. 9, 1922	0.20	0.92	Given small amounts of insulin
Aug. 28, 1922	0.21	1.61	
Sept. 3, 1922	..	1.54	
Oct. 20, 1922	..	2.56 2.30 1.80	During coma

* Analyses by Bloor's [11] total fat method. (Normal 0.75 per cent).

last few weeks he received small amounts of insulin but died in coma.

In Table II are given a few selected blood sugar and blood fat analyses which serve to show the general character of the blood changes.

Necropsy Report: Age 16 years. Necropsy one hour postmortem. Limited to upper abdominal incision. The body is that of a fairly well developed and nourished white boy. No edema. No jaundice.

Peritoneal Cavity: Surfaces smooth and shining. No free fluid.

Pleural Cavities: Negative.

Pericardial Cavity: Negative.

Heart: Normal size. No gross evidence of fibrosis. Valves negative. The coronary arteries are negative.

Lungs: Soft, crepitant except at bases, which are somewhat soggy. On section both bases are moist and reddish, with fluid in bronchi.

Spleen: Not remarkable.

Pancreas: Small.

Gastro-Intestinal Tract: Negative.

Liver: Not remarkable. The bile ducts are patent. The gall bladder is negative.

Kidneys: Negative.

Aorta: Scattered atheromatous plaques.

Anatomic Diagnoses: Hypostatic congestion of lungs, early arteriosclerosis.

Microscopic Findings.

Heart: Moderate amount of subepicardial fat. There is a slight increase of fibrous tissue.

Lung: Congested. A few polymorphonuclear leucocytes and red blood cells, together with endothelial leucocytes containing pigment, in alveoli. A few polymorphonuclears and lymphocytes in submucosa of bronchi. Moderate amount of carbon pigment beneath pleurae and about larger vessels and bronchi.

Spleen: Large vacuolated cells resembling endothelial leucocytes are present in the lymph nodules and in the splenic pulp. These cells are most prominent in the outer portions of the lymph nodules (Fig. 1). Some lymph nodules have reddish hyaline material in their centers, and a few contain scattered phagocytic endothelial leucocytes. Moderate degree of hyaline thickening of walls of arterioles.

With fat stains on formalin-fixed tissue preserved for three years, there appear to be two types of fat present (Table I, Case I). The first type, occurring in the larger globules found among the cells in the outer part of the lymph nodules as a rule, stains deep red with Sudan III and scarlet red, blue with Nile blue sulfate and brownish black with osmic acid. The second type occurs in smaller droplets in endothelial leucocytes both in the splenic pulp and to a less extent in the lymph nodules, as well as in the hyaline substance noted above and occasionally free in the neighborhood of small blood vessels. These droplets stain yellow-red with Sudan III, a faint red with scarlet red, very faint rose with Nile blue sulfate and pale gray with osmic acid. Both types of fat stain with the Smith-Dietrich method. With the polariscope when the slide is warmed, a few

anisotropic crystals are detected, which disappear with further heating but reappear on cooling. These findings are summarized in Table I (Case I).

Pancreas: Congested. Slight increase in connective tissue of islands. Moderate increase in interlobular connective tissue.

Liver: Fine vacuoles suggesting fat occur in the endothelium of the sinusoids. These stain red with Sudan III and scarlet red and brownish black with osmic acid. The liver cells are vacuolated especially about the central veins, and give similar staining reactions.

Kidneys: Not remarkable.

CASE II. No. 1794. White female. Onset of diabetes at 25 years of age. Body weight at onset 130 lbs. Duration of disease eight years. On June 6, 1920, blood sugar was 0.25 gm. per 100 cc. and blood fat 1.10 per cent. On May 21, 1921, blood sugar was 0.20 gm. per 100 cc. and blood fat 0.80 per cent. In June, 1922, she weighed when clothed 71 lbs. Under insulin treatment she gained weight and strength and in June, 1925, shortly before her death weighed 135 lbs. After breaking diet, she became suddenly ill with severe pain in the left chest and arm. She had five Stokes-Adams seizures, and died after a few hours from cardiac failure.

Necropsy Report: Age 33 years. Weight 130 lbs. Necropsy one hour postmortem. The body is that of a well developed and nourished white female. No edema. No jaundice. Abdominal fat layer one and one-fourth inches thick.

Peritoneal Cavity: Surfaces smooth and shining. No free fluid.

Pleural Cavities: Negative.

Pericardial Cavity: Negative.

Heart: Weight 315 gm. Epicardium negative. Considerable subepicardial fat. Apex of left ventricle yellowish gray, opaque and separated from normal myocardium by thin red zone. On section, lower two-thirds of interventricular septum is similar in appearance. A large thrombus is adherent to the endocardium. This entire region stains intensely red with scarlet red. In the endocardium just below the aortic ring are yellowish raised plaques which also surround the coronary orifices. The right coronary artery shows numerous atheromata; both branches of the left are tortuous and sclerosed. The first portion of the left barely admits a horsehair.

Spleen: Somewhat grayish. Only a small amount of pulp can be scraped away.

Pancreas: Weight 60 gm. Negative in gross.

Liver: Weight 1835 gm. Yellowish red. Bile ducts are patent. Gall bladder is distended with bile.

Kidneys: Weight 350 gm. Negative in gross.

Adrenals: Negative.

Bladder: Negative.

Genitalia: Negative.

Aorta: Numerous atheromatous plaques.

Anatomic Diagnoses: Infarct of heart; coronary sclerosis; and arteriosclerosis.

Microscopic Findings.

Heart: Moderate ingrowth of fat. Numerous hemorrhages into myocardium with polymorphonuclear leucocytes between the fibers. In several foci, muscle fibers have lost their striations and normal staining reaction, and have granular or hydropic cytoplasm. Numerous vessels are plugged with fibrin the meshes of which contain red blood cells and polymorphonuclear leucocytes. In one place there is marked fibrous thickening of the endocardium with hemorrhages beneath. Closely attached to the endocardium are masses of fibrin with red blood cells and polymorphonuclear leucocytes enmeshed. The coronary arteries show marked thickening of the intima due to collections of vacuolated and pigmented endothelial leucocytes. When stained for fat, the material in these cells is shown to be similar to that in the spleen.

Lungs: Edema. Carbon pigment is present beneath pleura and about larger vessels.

Spleen: Congested. Numerous plasma cells occur throughout. A large number of endothelial leucocytes filled with fat droplets are distributed throughout the pulp and are particularly abundant in the germinal centers (Fig. 2). Some of the cells contain numerous droplets (Fig. 3), while others are greatly distended by one large droplet (Fig. 4). The large ones are apparently due to the coalescence of droplets originally contained in endothelial leucocytes but set free by degeneration of the cells. The germinal centers are few and most of them contain masses of hyaline material. One contains a network of fibrin.

The results obtained with fat stains (on tissue fixed in formalin for five months) are summarized in Table I (Case II). In this spleen only one type of fat appears to be present. It stains yellow with Sudan III, faint yellow-red with scarlet red, very pale rose with Nile blue sulfate and very pale brown with osmic acid. Osmic acid appears to stain only the periphery of the globules, but they stain well with the Smith-Dietrich method. The polariscope reveals a few anisotropic crystals when the section is warmed, which disappear on further heating and reappear on cooling.

Pancreas: There is slight increase in the interlobular connective tissue. The islands appear to be much fewer than normal. Islands can be found showing varying degrees of sclerosis, from those apparently normal to practically complete disappearance of island cells.

Liver: The periportal areas contain numerous polymorphonuclear leucocytes, lymphocytes and plasma cells. Rarely a few of the liver cells at the periphery of the lobules are necrotic and surrounded by endothelial and polymorphonuclear leucocytes. Bile stasis is apparent in some portions. Endothelial leucocytes in the sinusoids are phagocytizing pigment, and some contain fine droplets. When stained for fat, these droplets are yellow with Sudan III and pale violet with Nile blue sulfate.

Kidneys: The glomeruli are markedly congested. The tubules are edematous. In Henle's loops the epithelial cells have lost all substance except for the nucleus and are greatly distended.

Aorta: There is very marked fibrous thickening of the intima in which are many endothelial leucocytes containing fat droplets. A few lymphocytes are also present. The vessels of the media are surrounded by an increased amount of fibrous tissue, with moderate infiltration by endothelial leucocytes. The droplets in the endothelial leucocytes when tested for fat are stained yellow with Sudan III and blue-black by the Smith-Dietrich method. With polarized light the droplets are found to be anisotropic.

Case III. No. 05–172. White male, 21 years of age. He was admitted to the hospital in deep coma and with a marked odor of acetone on the breath. No history could be obtained and death occurred in thirty hours. The urine showed a large amount of sugar and of diacetic acid, as well as a slight trace of albumen.

Necropsy Report: Age 21 years. Examination twenty-nine hours postmortem. The body is that of a well developed but poorly nourished white male. The significant findings in the necropsy may be summarized as follows.

Heart: The heart's blood on standing becomes covered with a creamy, bluish white scum. When the blood was placed in a cylinder this layer occupied the upper three-fifths of the column. The myocardium shows small scattered foci which are slightly darker than the surrounding tissue, translucent and slightly depressed.

Spleen: Weight 320 gm. On section it is dark red, firm, with markings distinct. A very small amount of pulp comes away on scraping.

Pancreas: Weight 45 gm. Negative in gross.

Liver: Weight 2045 gm. The capsule is smooth. The tissue is brownish red. The markings are indistinct. Occasionally foci throughout the liver, each involving several lobules, are paler and more yellowish.

Kidneys: Weight 360 gm. The capsule is stripped readily, leaving a smooth surface. On section the kidneys are reddish gray, congested and the markings are distinct.

Aorta: There are a number of opaque yellow raised plaques in the intima of the ascending portion of the arch. These are fewer in the remainder of the aorta, and they tend to cluster about the mouths of the intercostal arteries.

Microscopic Findings.

Heart: Sections of formalin-fixed material stained with scarlet red show fat droplets in the muscle fibers. The vessels contain much finely divided fat.

Spleen: There is moderate congestion. The meshes of the reticulum are filled with large foamy cells. When sections of formalin-fixed material are stained with scarlet red, these cells contain orange masses. The vessels contain much finely divided fat. In some of the nodules, the endothelial cells show evidence of proliferation. There is an increased number of eosinophiles.

Pancreas: Sections of formalin-fixed tissue stained with scarlet red show a considerable amount of finely divided fat in the epithelium of the acini and of the islands. The larger vessels show much finely divided fat.

Liver: There is considerable congestion. The nuclei are vacuolated, giving the typical appearance of glycogenic infiltration. The endothelial cells lining the sinusoids are somewhat enlarged and finely vacuolated. Sections stained with scarlet red show fine fat droplets evenly distributed throughout the liver cells and the endothelial cells.

Kidneys: The epithelium of the tubules is markedly vacuolated. In sections of formalin-fixed tissue stained with scarlet red, these vacuoles are seen to be filled with fat except in the region of Henle's loops. The character of the vacuolization here suggests glycogenic infiltration. There is also finely divided fat in the glomeruli and in the blood vessels.

Adrenals: The formalin-fixed sections stained with scarlet red show a marked deposit of fat toward the periphery.

Lymph Node: The sinuses are dilated and filled with vacuolated endothelial cells, some of which are in mitosis. In some of the lymph nodules there is an increased number of endothelial cells at the center. The endothelial cells contain finely divided fat.

Discussion

Although the significance of large numbers of lipoid-holding cells in the spleen is not clear, the rarity of the condition makes it seem worth while to record these three cases. So far as can be judged from the staining reactions, the lipoid is present in various forms and sometimes even varies in the same case, though cholesterol esters or related substances predominate. Thus in Case I, the larger droplets are presumably soaps or fatty acids among which, according to the reaction with osmic acid, are unsaturated fatty acids. The smaller droplets contain cholesterol esters.

In our first two cases, the long duration of the diabetes apparently has some relation to the condition. Two other cases of diabetes, about the same age as Case I, also died in coma. Their disease was of comparatively short duration, and the lipoid-containing cells were not found in the spleen, nor was there any evidence of lipemia, though complete necropsies were done. In Case II, the blood fat was abnormally high five years before death. The gain in weight from 71 to 135 pounds under insulin treatment and the amount of body fat found at necropsy suggest that more frequent analyses of

the blood would have revealed a more marked lipemia. In Case III, no history could be obtained, but the patient died in coma, with large amounts of sugar in the urine, and a striking lipemia was found at necropsy.

The spleen is not the only organ involved in this condition, though there the changes are more striking than elsewhere as a rule. Thus in Case I, lipoid is present in the spleen, the endothelial cells of the liver sinusoids and the intima of the aorta. In Case II, lipoid-containing endothelial cells occur in the spleen, liver, coronary arteries and aorta. The lymph nodes and bone marrow were not examined in either case. The fat is widely distributed in Case III, being present in the heart muscle, the parenchymal cells of the pancreas and liver and the renal epithelium, while the entire reticulo-endothelial system of the spleen, lymph nodes and liver contains lipoid material.

Oppenheimer and Fishberg [4] have stressed this involvement of the reticulo-endothelial system in lipemia and regard this group of cells as very important in relation to lipoid metabolism. Very similar changes to those in the human cases were produced in rabbits by Anitschkow [13] through feeding cholesterol dissolved in sunflower oil.

One of the puzzling features of this condition is the variation of the chemical constitution of the lipoid taken up by the cells of the reticulo-endothelial system. Of course the staining reactions are not entirely reliable criteria, but in several of the cases of this condition there is a marked difference in the reactions of the lipoid material in the circulating blood and that present in the reticulo-endothelial system. The selective absorption of cholesterol compounds and phosphatides seems not impossible.

Hyperplasia of lipoid-containing cells in the spleen has been observed in a few instances not associated with diabetes and lipemia. We encountered this condition in a negro 35 years old who died of an acute exacerbation of subacute pancreatitis. Smith [9] reported four instances of lipoid cell hyperplasia in infants and Siegmund [14] has reported one.

In the cases of diabetes with lipemia considered in this paper, there is another element of interest. Vascular lesions are very common. Whether these are merely xanthomas occurring in the intima of the vessels instead of the skin or definite atheromatous plaques is

not easily decided. The case reported by Oppenheimer and Fishberg [4] was a girl of 6 years, in whom yellowish patches occurred in the intima of the aorta and in the endocardium. Both patients reported by Lutz,[6] aged 53 and 36 years respectively, showed atheromatous patches in the aorta. The aorta of Smith's [9] case, a man of 22 years, contained atheromata. In the other reported instances the condition of the vessels was not noted. Atheromata were found in the aorta of our Case I, aged 16 years. Our Case II died of a cardiac infarct resulting from coronary sclerosis at the age of 33. The aorta also was markedly sclerosed. The lipoid present in the endothelial cells in the vascular lesions gave the same staining reactions as that in the cells in the spleen. Case III, 21 years of age, showed numerous yellowish, raised patches in the intima of the aorta.

Here then are five persons from 6 to 33 years of age, with lipoids in the blood that are taken up extensively by the cells of the reticulo-endothelial system, and all five show vascular changes.

There may well be a real danger in permitting patients to live on a diet of high caloric value with the aid of large amounts of insulin. The considerable intake of food above energy requirements, as shown by marked and often rapid gain in body weight, must result in large increase in the amount of lipoid in the circulation. While it is not proved that lipemia is harmful, these few cases suggest that there may be some relationship between it and arterial disease. In a condition such as diabetes, which seems to predispose toward arteriosclerosis, any factor which might tend to bring about vascular change should be avoided.

Summary and Conclusions

1. Three cases of diabetes mellitus with large lipoid-containing cells occurring in the spleen are reported and compared with eight others collected from the literature. Lipemia is present in all the cases except that of Fahr and Stamm.[10]

2. The lipoid material in the cells, so far as can be judged from staining reactions, consists of cholesterol esters or related substances, or phosphatides. Fatty acids or soaps may also be present.

3. The reticulo-endothelial system is probably involved in lipoid metabolism, and may perhaps selectively absorb cholesterol compounds or phosphatides.

4. Arteriosclerosis appears to be associated with this condition. Lipemia, produced by a high caloric diet, by a poorly balanced diet or by the general cachectic state of the tissues in severe diabetes, may predispose to atheromatous degeneration in the arteries.

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DESCRIPTION OF PLATES

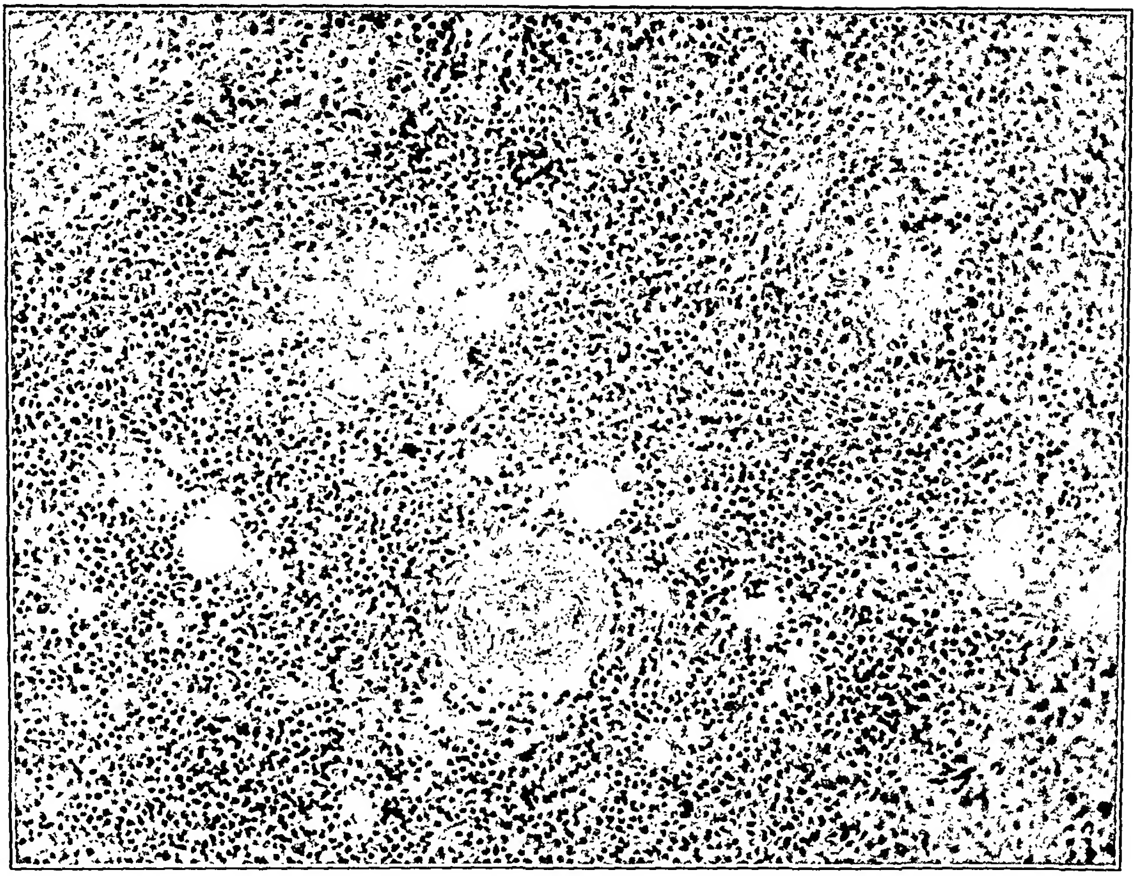
Plates 18–19

FIG. 1. Case I. Showing distribution of lipoid-containing cells in the spleen, and also hyaline material in the lymph nodules. X 100.

FIG. 2. Case II. Showing distribution of lipoid-containing cells in the spleen. X 100.

FIG. 3. Case II. Lipoid-containing cells in the spleen. X 500.

FIG. 4. Case II. Lipoid-containing cells and a large fat droplet formed by coalescence of smaller ones. X 500.



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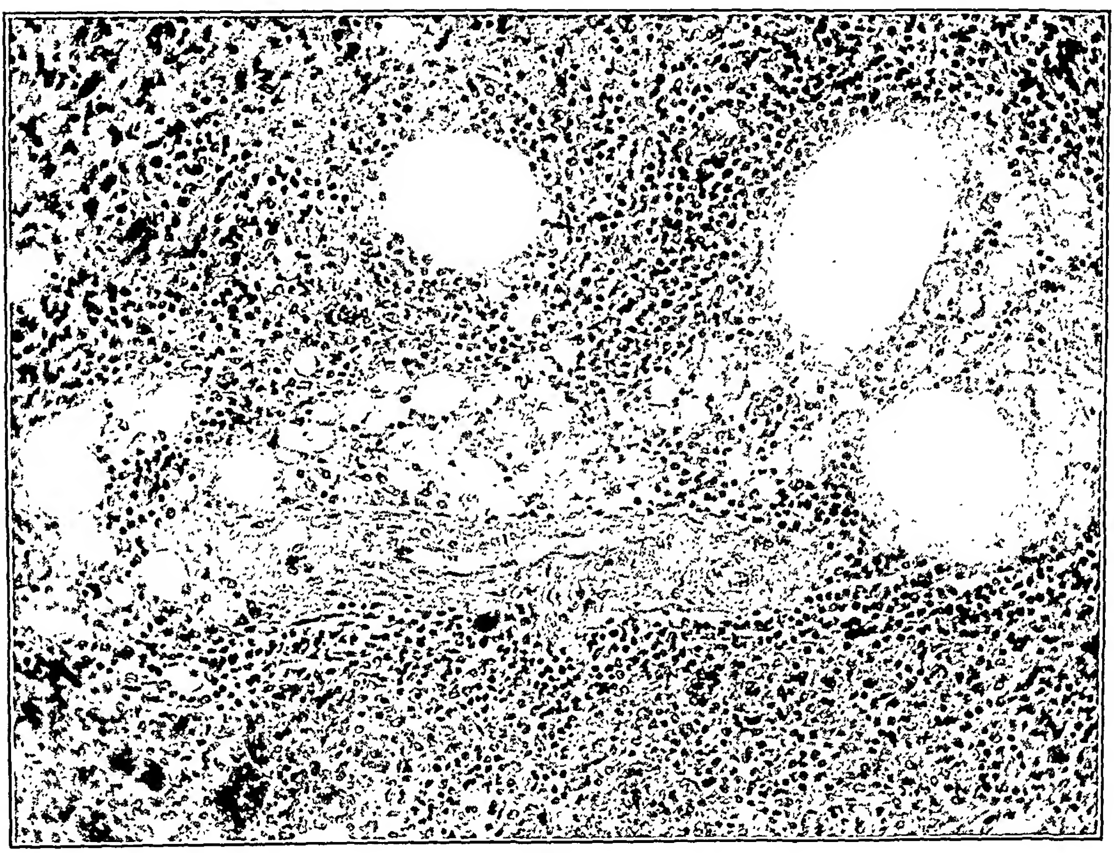

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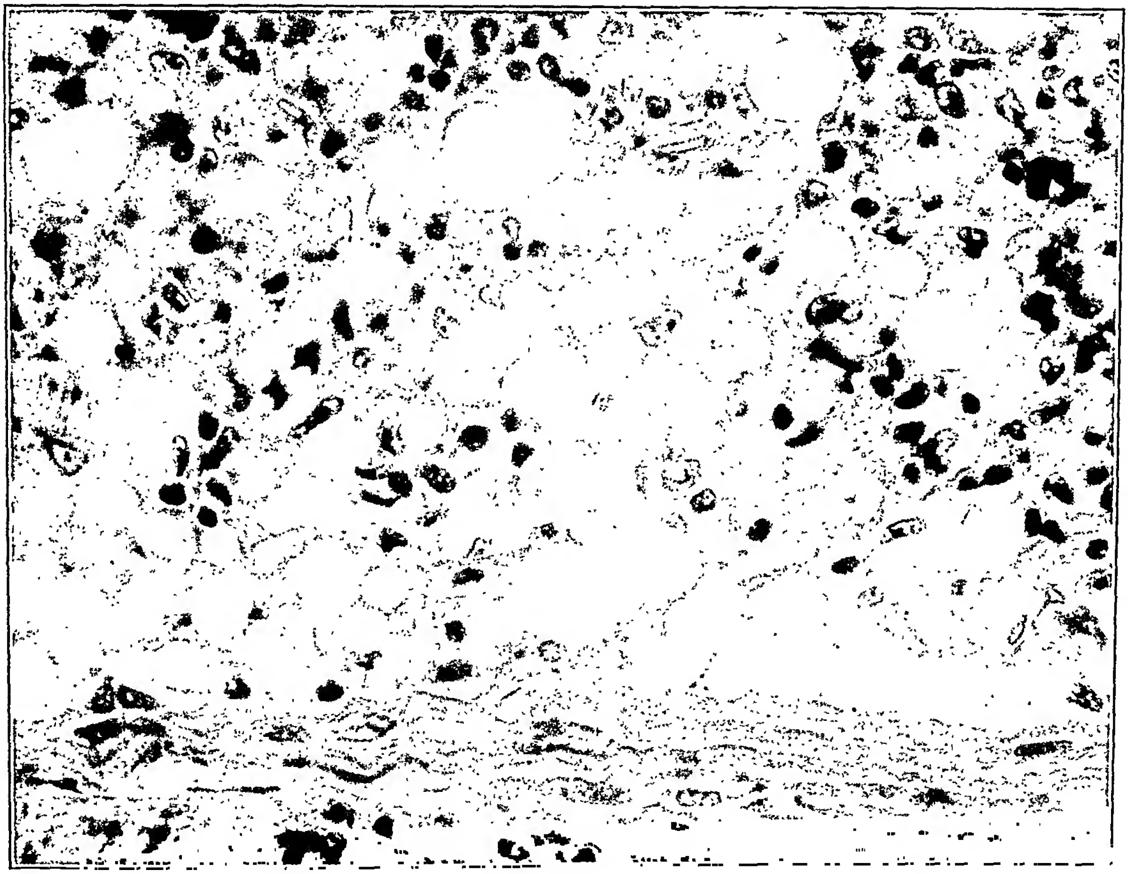
Warren and Root Lipoid-Containing Cells in the Spleen



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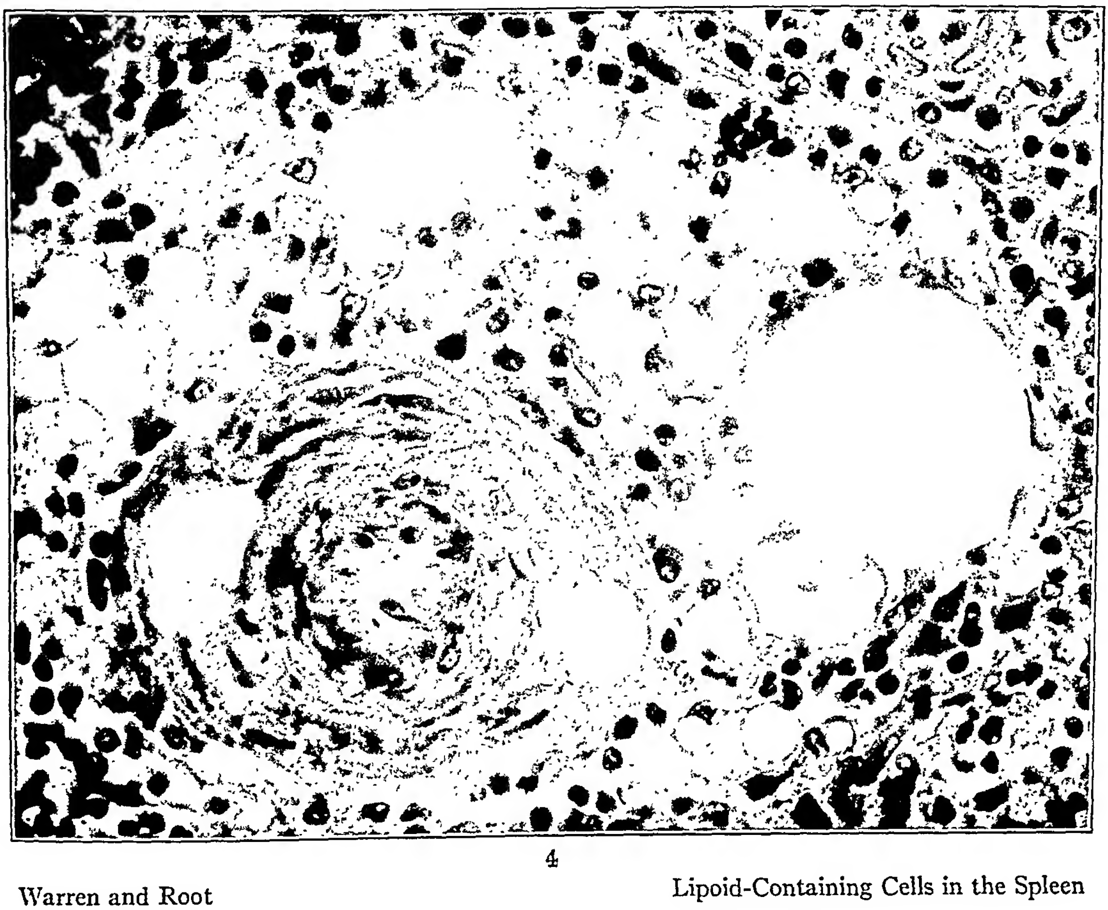

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Warren and Root Lipoid-Containing Cells in the Spleen

PRIMARY GELATINOUS CYLINDRICAL CELL CARCINOMA OF THE LUNG *

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Primary papillary cystadenoma of the lung is a rare tumor. Helly[1] and Löhlein[2] described such growths. Nicholson[3] found tumors of the lung of similar structure to be metastases from an apparently benign ovarian tumor. Cylindrical cell carcinoma of the lung with papillary and cystic formations has been described by several authors (Ribbert,[4] Briese,[5] Kretschmar[6] and Gordon[7]).

Helly[1] reported a distended emphysematous lung, studded with nodules of various sizes, containing granular stringy coagulated masses. The normal alveolar epithelium was replaced in the nodular areas with high columnar epithelial cells many of which were goblet cells. Adjacent alveoli were sometimes partially replaced with this type of epithelium. There was always a sharp demarcation between the normal alveolar epithelium and the high columnar cells of the tumor. The alveolar septa were not destroyed and the bronchi, bronchioles and blood and lymph vessels were not invaded. A clear line of demarcation between the tumor nodules and the surrounding tissue was not found. Helly considered this tumor to be a benign cystadenoma of the lung, starting from the transitional epithelium between the bronchioles and the alveoli. Löhlein[2] described a tumor in the right lower lobe of the lung, the size of a fist and gelatinous in consistence. Bronchi, bronchioles and lymph nodes were normal. The edge of the tumor mass was composed of cylindrical mucus-secreting epithelium which replaced the normal lining of the alveoli. In the center, the alveolar septa had been replaced by large cysts filled with tenacious mucous material. Pseudopapillary formations were observed. The transition between the neoplastic alveolar epithelium and the normal lining of the bronchi was not always distinct. Löhlein considered this to be a benign cystic tumor starting from alveolar epithelium. Sternberg men-

* Received for publication Nov. 19, 1925.

tioned in the discussion of this case a tumor of similar structure with brain metastases.

Nicholson [3] described tumors of the lung of similar structure, but he showed these to be metastases from a pseudomucinous cystadenoma of the ovary. Pick,[3] in a critical discussion of Nicholson's paper, Moise,[8] Adler [9] and others have discussed in detail the various interpretations that can be placed upon the origin of the primary tumors of the lung.

Report of Tumor

Some lung tissue was received from the surgical clinic (Auguste-Viktoria Krankenhaus, Berlin-Schöneberg) which seemed to show changes similar to those described by Helly, Löhlein and Nicholson. It was impossible to determine whether this tumor was primary or secondary from such a specimen. The patient died several months later and at necropsy it was found to be a primary lung tumor.

The surgical specimen was a piece of tissue the size of a hen's egg, rather dense with fibrous tissue and an adherent, grayish white, gelatinous substance. Cystic areas filled with opaque mucous material were scattered throughout the denser tissue.

Microscopically, this specimen is composed of fibrous tissue, fat and striated muscle. Elastic connective tissue stain brings out the elastic membrane of the pleura, which is split into layers by fibroblasts. Cystic areas visible in this connective tissue layer are lined with high columnar or goblet cell epithelium and contain material giving the reaction for mucin. True papillary formations can be observed. Delicate incomplete connective tissue septa between cysts show evidence of a confluence of several cysts. The loose connective tissue around some of these cysts is infiltrated with tumor cells and contains coagulated gelatinous extracellular substance (Fig. 1).

Adherent to the inner side of this fibrous layer is tissue which resembles, with low magnification, an emphysematous lung. Thickened connective tissue septa with perivascular round cell infiltration separate the lobules. The delicate alveolar septa are intact and the elastic tissue framework is shown by special staining to be normal in distribution. The distended alveoli are lined with regular, high cylindrical epithelium; goblet cells are present in places. In some few areas the normal cuboidal cells remain intact. The lining is

always in a single layer; transitional forms between the high columnar tumor cells and low cuboidal alveolar epithelium are not observed. Papillary growths into the distended alveoli or cysts are frequently seen. These alveoli are distended with a mucous mass which is a secretion of these lining tumor cells. Desquamated tumor cells are found in the lumen of the cysts, some with two or even more nuclei.

The ciliated cylindrical epithelium of the bronchi and terminal bronchioles is well preserved and not displaced by tumor cells. There is always a sharp line of demarcation between the deeper staining, ciliated epithelium of the bronchioles and the lighter staining, high cylindrical tumor cells of the alveoli (Figs. 2 and 3).

Résumé of Necropsy Findings

Mediastinum: Massed and adherent lymph glands on left side from hilum to level of first rib. These are hard and grayish white and on section show many cysts filled with tenacious, whitish mucus.

Right lung: Negative.

Left lung: Pleura firmly adherent. Lung solid and firm in consistence. No normal lung tissue found upon section. The lung is filled with grayish white, confluent nodules. Tissue between these nodules has a glass-like translucency and is composed of a delicate network with mucous cysts the size of a pin-head and larger. A tenacious mucus exudes from the whole cut surface. The bronchi are uniform in thickness and normal in appearance. The hilum glands are of the same consistence as the lymph glands of the neck.

Abdominal organs: Negative. The ovaries are entirely normal.

Microscopic Examination

The histologic findings are the same as those mentioned in the surgical specimen. The walls of some of the larger bronchi examined show an invasion by the tumor cells with complete destruction of the mucosa. The peribronchial lymph spaces and mucous glands are invaded and destroyed by the neoplastic growth. The hilum glands are completely replaced by tumor cells which form cysts and small and large groups of cells surrounded by mucous material. We consider this tumor to be a primary gelatinous cylindrical cell carcinoma of the lung.

We found primary carcinoma of the lung or the bronchi to have occurred in 33 of about 5000 necropsies between 1911 and 1921, or in 0.67 per cent. Older statistics mention a much lower percentage: Kaufmann [10] states 0.2 per cent in his textbook; Ewing,[11] 0.1 per cent; Briese,[5] 0.46 per cent; Barron,[12] 0.29 per cent during 1899 to 1921, but only for the years 1919 to 1921, 0.9 per cent. Recent publications of European investigators show an increase in the frequency of the lung carcinomas in the last years, similar to that observed by Barron for this country. Berblinger [16] noted a frequency of lung carcinomas in 6.6 per cent of all observed carcinomas in 1920, but of 11.11 per cent in 1923; Seyfarth [13] reported 6.23 per cent in 1920 and 15.5 per cent for the first half year of 1924; Lubarsch stated 5.4 per cent in 1920–21; Stähelin [17] found 2.19 per cent in 1900, against 4.9 per cent in 1924; and Kikuth [19] mentioned 1.7 per cent in 1897, but 9.4 per cent in 1923, adding that he could not state an increase of the absolute frequency of the carcinomas during this period in his material.

The indisputable increase of the lung carcinomas during the last twenty years, especially the last five, is the source of a lively discussion in the recent literature about its suspected causation. We may classify the different theories about the subject according to the type of the suspected causative factors and place them in three groups.

The first group may comprise the bacteriotoxic causes. A well known source of bronchial carcinoma is the metaplastic processes in the lining of old tuberculous cavities and syphilitic scars. The frequent occurrence of similar metaplasias in the bronchial mucosa, described by Askanazy [18] as precancerous lesions, after an influenza affection led several investigators (Moise,[8] Berblinger,[16] Läschke [20]) to suspect a correlation between the recent influenza epidemic and the marked increase of the lung carcinomas in the last years. But their conclusions show many weak points and are in the final analysis not very convincing. First, we miss a similar sudden increase of the lung carcinomas after the influenza epidemic in the years 1890 to 1894; second, the increase started in a minor degree already before 1920 as shown in the statistics of Stähelin,[21, 25] and Hampeln [14] comparing the data noted for the years before and after 1900 (0.1 to 0.2 per cent against 0.4 to 0.6 per cent); third, even the authors in favor of this theory could not prove the occurrence of a

previous "influenza," a diagnosis which was quite misused at that time, in the majority of their cases (Läschke[20] in less than 50 per cent). Other investigators of the same question (Gottstein, Stähelin, Kikuth, Hampeln) were unable to demonstrate this connection in a noteworthy percentage; fourth, the contrast in the male and female rates (Seyfarth 5.3: 1; Berblinger 4.25: 1 (for 1920 to 1924 only 2.7: 1); Kikuth 1.8: 1) is an important objection against this theory (Mathias[23]).

The causes classified in the second group are those of physical means. A trauma of the chest is too rare a cause of a lung carcinoma to have any relation to the increasing frequency of these tumors. Also the suspicion mentioned by Kikuth that the increasing use of X-rays for the treatment and diagnosis of thorax diseases might have some connection with the increase of the lung carcinomas seems to be unjustified because the frequency of the lung carcinomas does not correspond to the increase in the use of X-rays, and even Kikuth could not establish this connection in his material. Furthermore we may eliminate the inhalation of cigarette smoke as a causative factor for this increase, as stated by Fahr[23] who based his theory on the increased habit of cigarette smoking and the predominance of the affection in the male sex. Cigarette smoke may have only a contributory influence if any at all. A theory of more importance is supported by Hampeln.[14] He believes that a certain relation exists between the increased production of smoke and dust in the big cities which are substances causing by continuous inhalation a chronic irritation of the bronchial and lung epithelium, and the increasing frequency of the lung carcinomas. This theory, like that concerning the influenza epidemic, seems to be quite acceptable at first view, but the investigations of other authors (Dynkin,[21] Sachs,[25] Berblinger[16]) failed to substantiate this presumption. They were unable to state a higher frequency of carcinoma in pneumonoconiotic lungs than in other lungs. Schmorl[22] denies also a causative connection between the well known lung carcinoma of the Schneeberg miners with a primary pneumonoconiosis. He is rather convinced that these tumors are caused by the chemical effect of inhaled gaseous substances and suspects that arsenic compounds have to be made responsible for the frequent development of carcinomas in the lungs of these miners.

This explanation brings us to the last and third group of causa-

tive factors, those of chemical nature. We can eliminate from the list of the possible or suspected chemical factors which may be responsible for the increasing frequency, all those which have no generalized distribution and to which we are exposed to a certain degree for a period longer than the last twenty years. This consideration excludes the theory of Kraus[23] proposing a relation of the gas poisonings during the war with the lung carcinomas. By far the majority of the cases were never exposed to this injury. Furthermore, we can neglect the inhalation of acids and alkalis as unimportant in this question. A theory which fulfills the above mentioned conditions was expressed by Stähelin.[17] According to him the main factor responsible for the increase of the lung carcinomas in the last fifteen to twenty years is not the inhalation of smoke dust but the inhalation of dust containing chemical substances which possess a specific carcinoma-producing quality. He believes that the small tar and oil particles in the dust of tarred or oiled roads and the oxidation products of gasoline and benzol, daily inhaled in large amounts, are the causative factors for the increase.

His theory is based on the following viewpoints: (1) the tarring of the roads and the more general use of gasoline for automobiles date back about twenty years and have increased rapidly since that time; (2) tar products are recognized as carcinoma-producing substances in case of chronic application; and (3) carcinoma of the lung in rabbits and guinea-pigs was produced successfully by injection of tar products in the trachea (Kimura[24]). This theory is based on several very important facts and is worth examining on a broad experimental and statistical basis. Its practical importance is obvious in a country which has the most tarred roads and automobiles in the world.

Histologically there are three origins of the primary carcinomas of the lung: the epithelium of the bronchi, of the peribronchial mucous glands and the respiratory epithelium of the alveoli. Very briefly the characteristic features of these three types of carcinoma are as follows: carcinoma arising from the epithelium of the bronchi usually starts in the large bronchi, seldom in the bronchioles; it invades the lung along these bronchi from above downwards; and the cells are either cylindrical or squamous in outline. The former may form glandular cysts and at times mucus may be found.

The squamous cells are metaplastic bronchial epithelium. Car-

cinoma arising from the peribronchial glands invades the submucosa of the bronchi and replaces the bronchial mucosa extending down into the alveoli and adjacent structures; the lymph spaces and adjacent interlobular septa are invaded, with destruction of the lung architecture; and histologically the cells may resemble very closely those described in our case, but there is always a loss of the adenomatous structure in many places with solid and scirrhus carcinomatous changes possessing marked infiltrative power. Carcinoma arising from the pulmonary epithelium resembles grossly pneumonic infiltration. Histologically the cells are flat, cuboidal or cylindrical; the alveoli are lined or filled with tumor cells with sometimes a tendency to papillary formations; goblet cells with mucous secretion are characteristic for the cylindrical cell type; bronchial and bronchiolar epithelium does not show neoplastic changes; and the septa between the alveoli are not invaded by the tumor cells.

The carcinoma described in this report belongs to the primary pulmonary epithelial variety. The alveoli are well preserved and lined with tumor cells. The epithelium of the air passages is normal and does not participate in the carcinomatous growth. All adenomatous growths are lined by the same type of cells. There is no invasion of the alveolar septa.

Ribbert,[4] Briese,[5] Kretschmar [6] and Gordon [7] have each described a tumor similar to the one we are reporting here and have interpreted it as a primary carcinoma of the lung starting from the alveolar epithelium.

The question of single or multiple origin of such a tumor has been discussed by these authors. They concluded that there is either a diffuse genesis (Kretschmar, Gordon) or a multiple origin with the neoplastic changes occurring independently in many different foci (Briese, Ribbert). The multiple confluent nodules in the left lung of our case, without evidence of a primary focus, has lead us to the conclusion of the multiple origin of this tumor.

The mucus could probably serve as a medium of transporting carcinomatous cells from one alveolus to another within a limited area. The cells of typical adenocarcinomatous areas in the bronchial mucous membrane could be transported through the same medium and these areas would serve as foci for spreading to the alveoli of a wider area. We have mentioned that there was no invasion of the lymph spaces between the alveoli, and consequently

we think this was hardly an avenue for metastasis. The large lymph vessels along the bronchi and in the pleura showed adenocarcinomatous cysts. We could find no evidence of metastasis by the blood stream.

The tumors described by Helly and Löhlein were interpreted by them as benign adenomas based upon their non-metastasizing and non-destructive features. This can hardly be considered as conclusive evidence in view of the histologic nature of the neoplasms. It has been shown that the primary cylindrical cell carcinoma starting from the alveolar epithelium as described by Ribbert, Kretschmar, Briese, Gordon and by us does not destroy the alveolar structure of the lung and does not metastasize in all cases (Ribbert, Kretschmar, Gordon). Also we think that Pick's interpretation of Helly's tumor as a metastatic growth, because of its distribution throughout the lung in nodular formation, is hardly correct. We, as well as Ribbert and Briese, have observed the genesis of such adenocarcinoma from many different centers in the lung. Moreover, there was no clear line of demarcation between tumor tissue and lung tissue in these cases. Therefore, we conclude that these tumors are not benign adenomas but primary cylindrical cell carcinomas which started from the alveolar epithelium.

Finally, a short note may be included about the clinical difficulty in diagnosis between carcinoma and tuberculosis of the lung. It is known that sometimes a primary carcinoma of the lung starts in an old tuberculous cavity and that the physical and X-ray findings may be very similar in these diseases. But there is another source of wrong diagnosis. The finding of acid-fast bacilli in the sputum is usually considered as a proof for the tuberculous origin of a questionable lung disease. In the case just reported, acid-fast bacilli were found twice in sputum smears, hence the diagnosis of tuberculosis was made. The necropsy showed no evidence of tuberculosis of the lung. Therefore, the rods found were not tubercle bacilli but saprophytes (Muir and Ritchie [15]) which may be found in exudates from gangrenous processes in lungs. It is, therefore, necessary to judge such positive findings very cautiously, if there is any suspicion of a malignant growth in the lung.

Summary

1. The tumor in this case is a primary gelatinous cylindrical cell carcinoma of the lung, starting from the alveolar epithelium.

2. Two cases reported by Helly and Löhlein as benign adenomas of the lung must be considered as primary gelatinous cylindrical cell carcinomas of the lung starting from the alveolar epithelium.

3. The finding of acid-fast bacilli in the sputum of a patient with gangrenous processes in the lung due to a carcinoma may sometimes lead to the wrong diagnosis of pulmonary tuberculosis.

Note. The author wishes to express his appreciation and thanks to Dr. Lloyd Arnold for his help in preparing this paper.

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DESCRIPTION OF PLATE

Plate 20

Fig. 1. Cysts in the thickened pleura showing high cylindrical cells and stringy coagulated content with desquamated tumor cells.

Fig. 2. Lung. Papillary formation, with rosette-like groups of tumor cells in the alveolar spaces; and alveolar septa lined with high cylindrical cells.

Fig. 3. Bronchiole with normal cylindrical epithelium, round cell infiltrations in the interlobular septum, coal-dust pigment and emphysematous lung structure with well preserved alveolor septa which are lined partly with high cylindrical cells.

1

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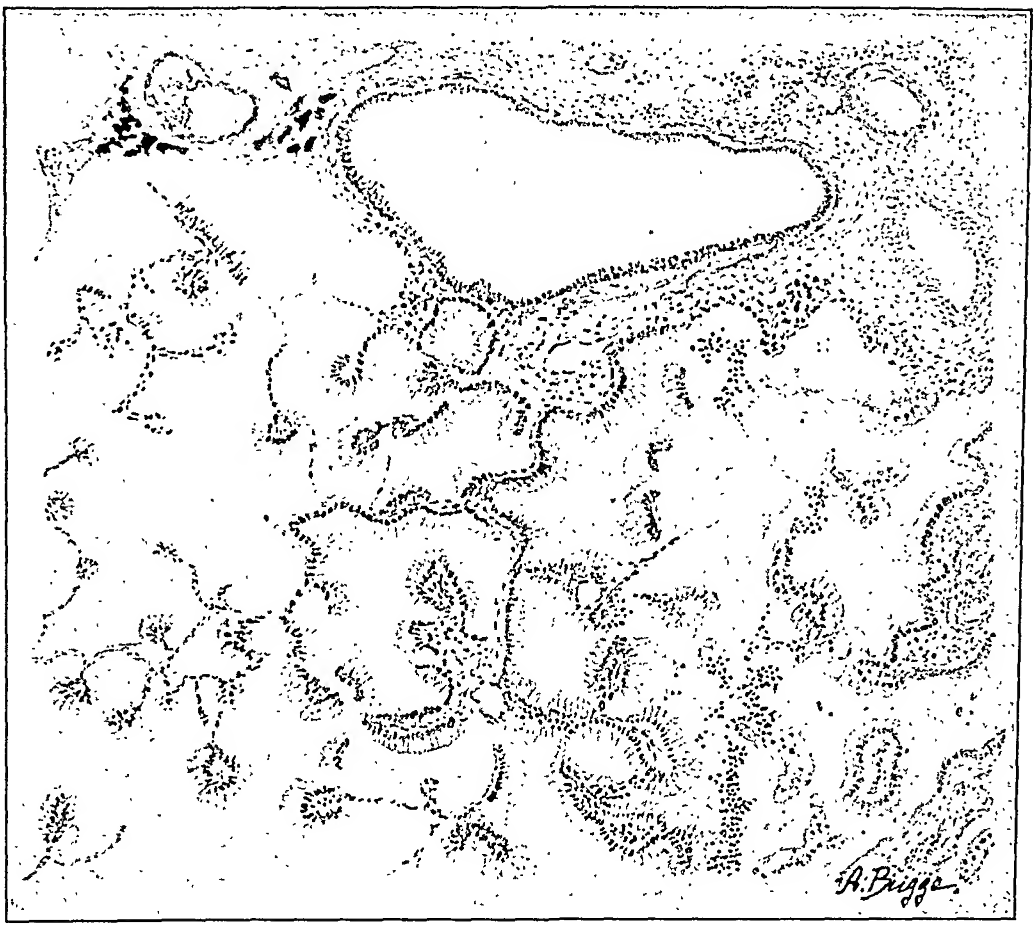

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Carcinoma of the Lung

A SUPRAVITAL STUDY OF LEUCOCYTES IN ALLERGIC STATES
A COMPARISON OF DELAYED AND IMMEDIATE INTRA-PLEURAL ANAPHYLACTIC REACTIONS *

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It has long been appreciated that the delayed skin reaction to proteins in rabbits (Arthus phenomenon) possesses some features in common with the tuberculin reaction in guinea-pigs in that both are delayed in onset and are characterized by similar macroscopic lesions, edema, erythema, induration and often necrosis. The authors, with Bradley,[1] have recently studied by supravital staining methods the behavior of reacting leucocytes during intra-pleural tuberculin reactions in guinea-pigs. It has seemed desirable to apply these same methods to the study of delayed and immediate anaphylactic skin reactions, the first being the type of allergy seen in rabbits and the second being the variety found in the guinea-pig.

Consequently a series of rabbits were sensitized by three intraperitoneal injections of horse serum at four day intervals, a total of 10 cc. of antigen. Twenty-one days after the initial injection, all of the rabbits gave strong precipitin reactions up to 1:10,000 dilution of antigen. Pleural exudates were induced in six animals, three sensitized and three controls, by intrapleural injections of 5 cc. of sterile hormone broth to which was added 0.3 cc. of horse serum. The cells of the resultant exudates were examined twenty-four hours later by supravital neutral red and Janus green staining in a constant temperature box. Cell counts are indicated in the following protocols.

Rabbit 774 (sensitized):

living polymorphonuclear neutrophiles, 41 per cent; dead, 44 per cent;
living lymphocytes, none; dead lymphocytes, 2 per cent;
living monocytes, none; dead, 8 per cent;
living clasmatocytes, none; dead, 5 per cent.

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RABBIT 1 (*control*):
living polymorphonuclear neutrophiles, 88 per cent; dead, none;
living polymorphonuclear basophiles, 1 per cent; dead, none;
living monocytes, 4 per cent; dead, none;
living clasmatocytes, 7 per cent; dead, none.

RABBIT 775 (sensitized):
living polymorphonuclear neutrophiles, 2 per cent; dead, 75 per cent;
living polymorphonuclear eosinophiles, none; dead, 6 per cent;
living lymphocytes, none; dead, 1 per cent;
living monocytes, none; dead, 13 per cent;
living clasmatocytes, none; dead, 3 per cent.

RABBIT 2 (*control*):
living polymorphonuclear neutrophiles, 87 per cent; dead, none;
living monocytes, 12 per cent; dead, none;
living clasmatocytes, 1 per cent; dead, none.

RABBIT 776 (sensitized):
living polymorphonuclear neutrophiles, none; dead, 90 per cent;
living lymphocytes, none; dead, 2 per cent;
living monocytes, none; dead, 8 per cent.

RABBIT 3 (*control*):
living polymorphonuclear neutrophiles, 90 per cent; dead, none;
living small lymphocytes, 1 per cent; dead, none;
living monocytes, 9 per cent; dead, none.

Examination of the peripheral blood of these rabbits revealed no dead cells.

Three guinea-pigs were sensitized to horse serum by doses similar to those used for the rabbits. Eighteen days after the last injection attempts were made to induce exudates in these and in three control guinea-pigs by intrapleural injection of 3 or 4 cc. of hormone broth. The following day all animals received additional intrapleural injections of 0.3 cc. horse serum to note its immediate effect on the cells of the preëxisting exudates. The results were not satisfactory. Two of the three sensitized guinea-pigs failed to show fluid; the third one gave a doubtful result, since many leucocytes in the exudates of both the sensitized and the corresponding control guinea-pig were injured, probably from over concentration of the dye but possibly from some normal toxic effect of the horse serum on guinea-pig cells. Two other control guinea-pigs, however, showed no dead cells in their exudates. To avoid any non-specific toxic action of horse serum, a second series of five guinea-pigs were sensitized by

two intraperitoneal injections of chemically pure crystalline egg albumen dialyzed free from ammonium sulphate. The total dose of antigen was 6 cc. of an approximately 10 per cent solution. Eighteen days after the last injection, pleural exudates were produced in the usual manner in four of the animals and twenty-four hours later 0.8 cc. of egg albumen solution was added to the preformed exudate. Two control animals were similarly treated. Cell examinations were made both before and after intrapleural injection of antigen. No dead cells were found before administering the antigen. The cell counts after antigen injection appear in the following protocols.

GUINEA-PIG 431 (sensitized):
living polymorphonuclear neutrophiles, 46 per cent; dead, none;
living polymorphonuclear eosinophiles, 10 per cent; dead, none;
living lymphocytes, 2 per cent; dead, none;
living monocytes, 42 per cent; dead, none.

GUINEA-PIG 1 (*control*):
living polymorphonuclear neutrophiles, 57 per cent; dead, none;
living polymorphonuclear eosinophiles, 4 per cent; dead, none;
living lymphocytes, 2 per cent; dead, none;
living monocytes, 34 per cent; dead, none;
living clasmatocytes, 3 per cent; dead, none.

GUINEA-PIG 432 (sensitized):
living polymorphonuclear neutrophiles, 66 per cent; dead, none;
living polymorphonuclear eosinophiles, 2 per cent; dead, none;
living monocytes, 28 per cent; dead, none;
living clasmatocytes, 4 per cent; dead, none.

GUINEA-PIG 2 (*control*):
living polymorphonuclear neutrophiles, 66 per cent; dead, none;
living polymorphonuclear eosinophiles, 1 per cent; dead, none;
living monocytes, 28 per cent; dead, none;
living clasmatocytes, 5 per cent; dead, none.

GUINEA-PIG 433 (sensitized):
living polymorphonuclear neutrophiles, 64 per cent; dead, 3 per cent;
living polymorphonuclear eosinophiles, 4 per cent; dead, none;
living lymphocytes, 2 per cent; dead, none;
living monocytes, 17 per cent; dead, none;
living clasmatocytes, 10 per cent; dead, none.

GUINEA-PIG 3 (*control*):
living polymorphonuclear neutrophiles, 67 per cent; dead, none;
living polymorphonuclear eosinophiles, 2 per cent; dead, none;
living monocytes, 30 per cent; dead, none;
living clasmatocytes, 1 per cent; dead, none.

In order to verify the sensitization, guinea-pig 432 was given 1 cc. of antigen intravenously after the pleural tap had been made; the animal died a typical anaphylactic death in about five minutes. Guinea-pig 433 died an anaphylactic death about forty-five minutes after receiving the antigen intrapleurally; necropsy showed distended, rigid lungs, a heart still beating and small gastric hemorrhages; microscopically lungs were markedly edematous. The cells of the exudates of both of these animals were alive and motile three hours after the death of the guinea-pig and of course were all that time in contact with antigen.

One guinea-pig received 0.3 cc. of antigen in 4 cc. of hormone broth in the right pleural cavity in order that any delayed reaction might be studied. In twenty-four hours the fluid contained only living cells. The cell count was as follows:

living polymorphonuclear neutrophiles, 77 per cent; dead, none;
living polymorphonuclear eosinophiles, 1 per cent; dead, none;
living small lymphocytes, 1 per cent; dead, none;
living monocytes, 16 per cent; dead, none;
living clasmatocytes, 5 per cent; dead, none.

The question was then raised as to whether the rabbits sensitized to horse serum would show any immediate reaction. Consequently, using the same rabbits employed for the study of the delayed reaction, the precipitin titer was brought back to a high level (1:10,000) by two additional injections of horse serum intraperitoneally. Pleural fluids were produced with hormone broth and in twenty-four hours 1 cc. of horse serum was added to the exudate. The rabbits were tapped twenty to thirty minutes later. One animal gave no fluid; the other two yielded an exudate containing only living cells.

Rabbit 774 (sensitized):

living polymorphonuclear neutrophiles, 42 per cent; dead, none;
living polymorphonuclear basophiles, 1 per cent; dead, none;
living polymorphonuclear eosinophiles, 1 per cent; dead, none;
living lymphoocytes, 1 per cent; dead, none;
living monocytes, 23 per cent; dead, none;
living clasmatocytes, 32 per cent, dead, none.

Rabbit 775 (sensitized):

living polymorphonuclear neutrophiles, 50 per cent; dead, none;
living lymphocytes, 6 per cent; dead, none;
living monocytes, 15 per cent; dead, none;
living clasmatocytes, 26 per cent; dead, none;
living serosal cells, 3 per cent; dead, none.

Summarizing the above results, it will be seen that in rabbits the leucocytes are killed after contact with the antigen for twenty-four hours but not after a shorter period, *i.e.*, thirty minutes. In guinea-pigs, on the other hand, the leucocytes are not injured at either period. The differences in the results in these two animals are interesting and significant in view of the difference in their skin reactions. As is well known, a sensitized guinea-pig gives an immediate skin reaction on reinjection of the antigen intradermally; this skin reaction is transient and causes no lasting injury such as necrosis. The guinea-pig under ordinary conditions, at least, does not give a delayed skin reaction. A sensitized rabbit, on the other hand, never gives an immediate skin reaction but does give a delayed skin reaction which is more than transient and in a highly sensitized animal leads to definite injury such as necrosis. The cause for the variation in the two animals is not yet clear; it has been suggested by some that the difference is dependent on the inequality in amount of circulating antibodies, for in guinea-pigs the titer of these is usually low in comparison to the titer in rabbits. However, whatever the explanation of this difference in type of reaction may be, in the above experiments an analogous condition as regards their leucocytes apparently holds true, *i.e.*, the leucocytes of the guinea-pig are uninjured by antigen whereas those of the rabbit are killed. This apparently is a fundamental difference and may throw some light on the dissimilarity in the types of skin reactions, for it seems improbable that these cells alone, the leucocytes, should be the only cells that become sensitive to the antigen. Here again the presence of a large amount of circulating antibody can conceivably be the cause of this difference; further work along this line is in progress.

On comparing these results with those obtained by the authors in working with tuberculin sensitization in guinea-pigs, a striking similarity will be noted. In a tuberculin reactive guinea-pig the leucocytes are killed on contact with tuberculin for twenty-four hours. The skin tuberculin reaction in a guinea-pig is of course a delayed reaction and the analogy with a rabbit sensitive to horse serum, giving a delayed reaction, whose leucocytes are killed by the antigen, is evident.

If we consider the death of leucocytes on exposure to antigen as due to some definite inherent cause dependent on factors other than circulating antibody content, then we are faced with the following

situation. In protein sensitized guinea-pigs that give only an immediate reaction, the leucocytes are uninjured by antigen. In protein sensitized rabbits that give only a delayed reaction, the leucocytes are killed by the antigen. In guinea-pigs reactive to tuberculin, that give only a delayed reaction, the leucocytes are killed by the antigen. How leucocytes of tuberculin sensitive rabbits behave in contact with tuberculin is a question which will be dealt with in the near future.

Two small histologic details, although wholly apart from the general subject of this communication, nevertheless deserve reporting. The first of these concerns the question of phagocytized polymorphonuclears. The writers had always assumed from study of fixed preparations that when once a polymorphonuclear had been taken up by a monocyte or clasmatocyte, the phagocytized cell died. Probably in most instances this is true although death is not immediate and many living polymorphonuclears are engulfed to be destroyed later. On one occasion during the present study, a phagocytized polymorphonuclear neutrophile was observed to free itself completely from a clasmatocyte and to escape. The other point of note may be described briefly as follows.

While studying the pleural effusions of rabbits for evidence of an immediate type of reaction, we observed what appeared to be direct cell division in two polymorphonuclear leucocytes; one of these divisions was seen too late to follow the method, but the other was observed in fair detail: a polymorphonuclear neutrophile with a triple nuclear mass was seen to segment partially, one of the nuclear masses passing through the constricted zone and two remaining behind; granules could be observed passing through the constricted portion which gradually became greatly attenuated and finally ruptured, the cells moving off in opposite directions. No evidences of mitotic phenomena were seen. So far as we are aware, neither of the above observations has hitherto been recorded although no survey of the literature has been made.

Summary and Conclusions

In the delayed intrapleural anaphylactic reaction in rabbits sensitive to horse serum, reacting leucocytes are largely killed.

In this respect, the reaction parallels the intrapleural tuberculin reaction in guinea-pigs.

Attempts to induce an immediate intrapleural anaphylactic reaction in rabbits resulted in no apparent cell injury.

In sensitized guinea-pigs no cell injury either immediate or delayed was observed when antigen was introduced into the pleural cavity.

Evidence gleaned from this study accords well with facts already known about delayed and immediate anaphylaxis.

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VALVULAR DISEASES OF THE HEART
WITH SPECIAL REFERENCE TO THE PATHOGENESIS OF OLD VALVULAR DEFECTS *

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The primary object of this investigation is to trace the development of old valvular defects. This involves a detailed study of all forms of acute endocarditis, especially with reference to the manner of healing. We have available for study 280 cases of valvular heart disease, not including those of syphilitic origin.

We have classified the valvular diseases as follows:

I. Rheumatic endocarditis
  1. acute
  2. recurrent or chronic

II. Bacterial endocarditis
  1. acute $\begin{cases} a.\ \text{primary} \\ b.\ \text{secondary} \end{cases}$
  2. subacute

III. Old valvular defects
  1. inflammatory $\begin{cases} \text{Group 1} \\ \text{Group 2} \end{cases}$
  2. calcified nodular — Group 3
  3. congenital

IV. Syphilis of the aortic valve

I. RHEUMATIC ENDOCARDITIS

1. ACUTE RHEUMATIC ENDOCARDITIS. A summary of eighteen cases of this condition is given in Table 1. The diagnosis is based upon the gross appearance of the lesions on the valves. Although there are no fundamental differences, it is convenient to distinguish

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rheumatic and bacterial vegetations. The former are small, firm, discrete or fused to form a narrow ridge, and translucent to pale red in color. Their consistence is such that they do not become detached to form emboli. Bacterial vegetations are relatively large and vary in color from red to white. They are of soft consistence and portions readily break off to form emboli. The microscopic differences will be discussed later. Transition forms are occasionally seen, and frequently both types of vegetations are found in the same heart or even on the same leaflet. The cases classed as rheumatic are those in which no typical vegetations of bacterial type are found.

Seven of the eighteen patients were suffering with multiple acute arthritis at the time of death and were therefore typical clinical cases of acute rheumatic fever. Five others had clinical signs of systemic infection but had no arthritis. In the light of the postmortem findings these may also be considered acute rheumatic fever, since it is well known that rheumatic endocarditis may exist without arthritis. The case of chorea belongs definitely to the rheumatic group.

But there are five cases in which the endocarditis was an accidental postmortem finding. In Nos. 24-364 and 25-411 there are no clinical data. In the case of acute poliomyelitis, the rheumatic lesion may be interpreted as an independent terminal infection; but in the two cases of acute endometritis there is a strong suggestion that the endocarditis was produced by the organisms responsible for the uterine infection. Rheumatic endocarditis does occur secondary to infectious processes though much less frequently than the bacterial type.

Acute pericarditis was present in every case that died primarily of rheumatic fever, and this complication seems to be largely responsible for death in the acute stage.

Coincident with the formation of the vegetations there is a diffuse inflammation of the marginal part and often of the entire leaflet. The leaflet shown in Fig. 1 is opaque throughout; its capillaries are greatly dilated, and there are several small hemorrhages. Microscopically this leaflet shows an extensive lymphocytic exudate throughout. This diffuse distribution of the inflammation in the acute stage is important in the interpretation of the old valve defects to be discussed later on.

TABLE 1

Acute rheumatic endocarditis

Number	Age in years	Sex	Duration	Acute arthritis	Sepsis without arthritis	Acute pericarditis	Valves involved	Aschoff bodies	Cause of death	Glomerulo-nephritis
10-92	14	F	2 wks.	–	..	–	mitral	+	chorea	–
15-83	28	F	several months	+	..	+	mitral	–	rheumatic fever	?
16-272	23	M	1/12	–	+	+	mitral	+	rheumatic fever	–
16-326	18	M	?	–	..	–	mitral	–	acute poliomyelitis	?
17-106	4	F	3 wks.	+	..	+	mitral	–	rheumatic fever	–
20-319	3½	M	3 wks. +	+	..	+	mitral, aortic	–	rheumatic fever	?
22-185	6	F	2 mos.	+	..	+	mitral, tricuspid	+	rheumatic fever	?
22-209	28	F	3 days	–	..	–	aortic	–	acute endometritis	–
22-595	9	F	3 wks.	–	+	+	mitral, aortic, tricuspid	+	rheumatic fever	?
23-354	12	M	2 mos. +	–	+	+	mitral	+	rheumatic fever	?
24-1a	15	F	3 mos.	+	..	+	mitral, aortic	+	rheumatic fever	?
24-275	34	M	3 wks.	+	..	+	mitral	+	rheumatic fever	–
24-364	40	M	?	–	..	–	mitral	+	undetermined	–
25-163a	16	M	3 mos.	–	+	+	mitral, aortic	+	rheumatic fever	?
25-171	19	M	2 mos.	+	..	+	mitral, aortic, tricuspid	+	rheumatic fever	?
25-207	31	F	8 days	–	..	–	mitral	–	acute endometritis	–
25-319	3 mo.	M	3 days	–	+	–	mitral	–	rheumatic fever, meningocele	–
25-411	23	F	?	–	..	–	mitral	+	acute lysol poisoning	–

2. Recurrent or Chronic Rheumatic Endocarditis (Table 2). In this group all hearts are included that show acute lesions of rheumatic type along with thickened scarred leaflets. It is not possible to make an anatomic distinction between chronic and recurrent forms but this may sometimes be done clinically.

Clinical Features. In this group of eighteen patients, thirteen gave a definite history of one or more attacks of acute arthritis, and of these, two had arthritis at the time of death. The history was incomplete in four cases.

Sixteen patients were suffering from chronic cardiac disease, and of these, five died apparently of cardiac failure without any clinical signs of a terminal infection, two of lobar pneumonia, one from a bleeding gastric ulcer and one from an anesthetic. Of the remaining seven, five had clinical findings suggesting an active infection, but did not have arthritis, and two had typical rheumatic fever at the time of death.

The two patients that had no signs of chronic cardiac disease died suddenly, one from a gunshot wound, the other from an undetermined cause.

A case of this group may be recognized clinically when the symptoms and signs of an active rheumatic infection are present in association with the evidences of an old valvular defect. These conditions were fulfilled in only six cases, and even these were difficult to distinguish clinically from subacute bacterial endocarditis.

Gross Pathology. The vegetations are identical grossly with those of the acute rheumatic type described above. In nine hearts, only the previously thickened valves showed acute vegetations; but in three hearts the vegetations were found on the normal valves and not on the defective ones. In six hearts both kinds of valves showed vegetations, and in nine hearts, therefore, acute lesions were found on valves not previously diseased. In a recurrent infection there seems to be a definite tendency for more valves to become involved.

The appearance of the affected thin leaflets corresponds entirely to the acute rheumatic type; but on the thickened leaflets the vegetations are usually not prominent and are easily overlooked (Fig. 2). The situation of the vegetations is the same as in the acute type.

Considering both acute and chronic changes, the mitral valve was affected in 17 instances, the aortic in 14, the tricuspid in 6 and the pulmonary in 1. The mitral alone was involved in 3 cases, but no

other valve was affected singly. There is evidently a greater tendency for the original infection to attack the mitral value. (Table 2, mitral valve defect 17, aortic valve defect 8) (*cf.* also Table 1, mitral 19, aortic 7).

Structure of Rheumatic Lesions.[1] The stages preliminary to the appearance of the vegetations are not known with certainty since the disease is recognized only when they are present. In association with the smallest vegetations there is already a widespread inflammation throughout the marginal part of the leaflet. In 25–319 (Table 1) the clinical duration was only three days and the vegetations were few and very small. Portions of the leaflets between vegetations show many fibroblasts and polymorphonuclear leucocytes, as well as edema. In well developed cases, the margins of the leaflets apart from the vegetations may show a structure such as is shown in Fig. 3, *viz.*, many large fibroblasts, a few lymphocytes and polymorphonuclears, and an intact surface endothelium.

The smallest vegetations vary somewhat in structure, depending upon the relative proportion of fibroblasts, hyalin, and serous and cellular exudate. The most common type is shown in Fig. 4 (right-hand side). There is a dense collection of fibroblasts near the surface with a little hyalin at the surface. A few polymorphonuclears and lymphocytes are present. On the left-hand side of Fig. 4, the vegetation contains a large amount of hyalin. The endothelium is detached over the central part of the vegetations.

In some vegetations hyalin is a very prominent constituent (Fig. 6). It forms in masses within the leaflet and breaks through the endothelium.

A typical well formed vegetation is elevated from the surface and sometimes pedunculated (Fig. 7). The core is formed chiefly of fibroblasts, but there is a varying amount of serous exudate (edema) and cellular exudate (lymphocytes). Small capillaries are always present. There is a surface layer of hyalin under which the fibroblasts are somewhat more closely packed than elsewhere (Fig. 8). The surface endothelium is largely detached over the hyalin. In some places small platelet thrombi form on the rough surface (Fig. 5). Underneath the vegetation there is a marked inflammatory reaction, characterized by extensive perivascular lymphocytic infiltration and numerous small fibroblasts.

The vegetations seem to be merely localized swellings on the leaflet

TABLE 2

Recurrent rheumatic endocarditis

Number	Age in years	Sex	Duration of cardiac symptoms	Previous attacks of acute arthritis *	Arthritis at time of death	Sepsis at time of death	Valves previously involved	Valves with acute lesions	Weight of heart in grams	Aschoff bodies	Cause of death	Acute pericarditis	Old pericardial adhesions	Glomerulonephritis
13–74	16	F	8 mos.	5 yrs., 4 yrs., 3 yrs., 2 yrs., 8 mos.	–	–	mitral, I_2	aortic	enlarged	+	lobar pneumonia, C. F.	–	–	–
13–142	14	F	5 yrs.	5 yrs., 3 mos.	+	..	mitral, I. S.	mitral, aortic, tricuspid	400	+	C. F., rheumatic fever	–	+	–
15–377	47	M	2 yrs.	2½ yrs.	–	–	aortic, I_1 mitral, I_1	mitral	1061	–	C. F.	–	+	?
17–227	56	M	5 yrs.	many attacks	–	–	mitral, I_1 aortic, I_1	mitral	475	–	C. F., lobar pneumonia	–	–	–
18–123	35	M	3 wks.	?	–	–	aortic, I_3, mitral, S. I.	mitral	800	+	C. F.	–	–	–
18–204	21	M	0	?	–	–	mitral	mitral	350	–	gunshot, immediate death	–	–	–
20–153	37	F	6 mos.	6 mos.	+	..	mitral, I. S.	mitral, aortic tricuspid	410	+	rheumatic fever, C. F.	–	–	–
20–163	15	M	3 yrs.	–	–	+	mitral, S_3	tricuspid	250	–	C. F., acute tonsillitis	–	–	–
21–194	35	F	1 yr.	4 yrs.	–	+	mitral, S. I., aortic, I_1	mitral	440	–	C. F., sepsis	–	–	–

21–253	23	M	7 mos.	7 mos.	–	–	aortic, I_2, mitral, S. I.	mitral, tricuspid	575	+	C. F.	–	–	–
22–28	14	F	1 mo.+	2 yrs., several later	–	+	mitral, I. S.	mitral, aortic	400	+	acute pericarditis, C. F.	+	–	–
23–153	40	M	8 yrs.	8 yrs.	–	–	mitral, S_2, aortic, I. S., tricuspid, S_2, pulmonary, I. S.	mitral, aortic, tricuspid, pulmonary	600	–	C. F.	–	–	–
23–161	48	F	1 mo.	1 mo.	–	+	mitral, S. I.	mitral, aortic	550	+	C. F., acute pericarditis	+	–	?
23–480	35	M	?	2 mos.	–	–	aortic, I_3, mitral, I_1	aortic, mitral	800	+	C. F.	+	–	–
24–519	23	F	?	?	?	?	mitral, S. I.	mitral	325	–	?	–	–	–
24–2a	15	F	1 yr.	4 yrs.	–	+	mitral, I_2	mitral, aortic	300	+	C. F., rheumatic fever	+	–	?
25–112	36	M	several yrs.	?	–	–	aortic, I_3	tricuspid	800	+	C. F., gastric ulcer hemorrhage	–	–	?
25–621	41	M	10 yrs.	1 mo.	–	–	mitral, S_3	mitral	500	–	anesthetic, C. F.	–	–	?

Legend: C. F. = cardiac failure. S = stenosis. I = insufficiency. S. I. = chiefly stenosis. I. S. = chiefly insufficiency.
The degree of stenosis or insufficiency is indicated by the subnumbers 1, 2, 3.

* Months or years preceding death.

due to an uneven intensity of the inflammatory reaction. What appears grossly to be a single vegetation is usually found microscopically to be a series of small confluent vegetations (Fig. 5).

Different degrees of intensity of the inflammatory reaction are to be noted. In 25–163a the marginal part of the leaflet is involved throughout its cross-section and there is a hyaline layer on both surfaces (Fig. 9). The fibroblasts are large and numerous and some multinucleated forms are seen (Fig. 10). There are many mitotic figures.

The fibroblasts are the most conspicuous cells in a rheumatic lesion. They develop from the fixed connective tissue cells of the leaflet. They may appear as spindle-shaped, branched or rounded with abundant cytoplasm. The reaction is the same as in proliferative inflammation elsewhere. The cell body enlarges and becomes rounded. Cell division occurs repeatedly. Frequently multinucleated fibroblasts are seen. In one valve, 25–163a, a number of typical Aschoff nodules are found.[2] As far as one may judge from transition forms, the multinucleated cells, separate or in Aschoff nodules, are derived from fibroblasts.

Rheumatic inflammation is chiefly proliferative in character but it is not uncommon to find a few polymorphonuclears in the earlier lesions; and small lymphocytes are numerous, especially in the leaflet below the vegetation. The lymphocytes tend to collect around the capillaries. Areas of serous exudate (edema) are often seen in the vegetations and elsewhere, and this is one cause of the swelling.

Dense hyaline material is found constantly on the surface of fresh vegetations. Some small vegetations consist chiefly of hyalin (Figs. 4 to 6). It may also be found deep within the substance of the leaflet apart from vegetations (Fig. 11). It seems to be chiefly a coagulated exudate and not a product of tissue disintegration. When the hyalin breaks through the endothelium, platelets may accumulate upon it (Fig. 5). A platelet thrombus cannot be distinguished from this hyaline material except by its position. Fibroblasts may be found scattered through the hyalin but they are usually not so numerous as elsewhere.

The reaction in the leaflet apart from the vegetations is very important in determining the subsequent effects upon the valve. The marginal part of the leaflets, especially of the mitral and tricuspid

TABLE 3

Acute primary bacterial endocarditis

Number	Age, in years	Sex	Duration	Arthritis, time before death	Valves involved	Acute pericarditis	Aschoff bodies	Glomerulonephritis
11–128	42	M	2 days	?	mitral	–	–	–
14–209	21	F	1 wk.	?	mitral	–	–	mild acute exudative
14–232	55	F	5 wks.	–	mitral	–	–	–
15–159	54	M	10 days	?	mitral	+	–	–
15–391	43	M	3 wks.	?	aortic	–	–	–
16–54	35	M	2 wks.	–	mitral	–	–	–
16–128	19	F	5 days	?	mitral	–	–	–
17–31	19	M	3 wks.	–	aortic	–	–	glomerular abscess
19–197	34	F	6 days	?	mitral, aortic	–	–	–
20–2	18	M	5 wks.	?	aortic	–	–	–
21–124	78	M	4 wks.	1 mo., still present	mitral, aortic	–	–	–
22–148	19	F	4 wks.	1 mo.	aortic	–	–	embolic
17–213	43	F	2 wks.	2 wks., still present	all four	+	–	embolic

TABLE 4

Acute secondary bacterial endocarditis

Number	Age, in years	Sex	Duration	Arthritis, time before death	Valves involved	Acute pericarditis	Aschoff nodules	Primary disease	Glomerulonephritis
10–59	7	F	5 days	–	mitral	+	–	otitis media	–
12–18	33	F	10 days	–	mitral	–	?	acute endometritis	–
13–16	31	F	?	?	mitral	–	–	tuberculosis with cavities	(amyloid)
14–255	39	M	4 wks.	6 yrs.	aortic	–	–	lobar pneumonia	acute diffuse
16–89	30	M	2 wks.	4 yrs.	aortic, mitral	–	–	erysipelas	subacute diffuse
16–308	23	F	1 wk.	?	mitral	–	–	acute endometritis	–
16–358	62	M	?	?	mitral	–	–	postoperative peritonitis	–
16–381	23	F	a few days	?	mitral	–	–	acute endometritis	–
17–233	38	F	?	has chronic arthritis	mitral	+	–	abdominal tumor	–
17–248	38	F	a few days	?	mitral	–	–	acute endometritis	–
18–39	39	M	?	?	mitral	–	–	pernicious anemia	–
18–53	47	M	?	?	aortic	–	–	carcinoma of stomach	–
19–94	46	F	9 days	?	mitral	+	–	postoperative peritonitis	–
20–209	56	M	?	?	mitral, aortic	–	–	peritonitis (carcinoma of stomach)	–

20-267	25	F	?	?	mitral, tricuspid	–	–	acute endometritis	acute diffuse
20-388	38	F	?	many in past 2 yrs.	mitral	–	–	leukemia	–
20-428	65	M	1 wk.	?	mitral	–	–	influenzal pneumonia	–
21-150	21	F	4 wks.	?	mitral	–	–	acute endometritis	–
21-237	36	F	3 wks.	?	aortic	–	–	acute endometritis	–
21-307	54	M	10 days	2 mos.	mitral	–	–	primary hypertension	–
22-134	46	F	9 days	?	mitral	–	–	influenzal pneumonia	–
22-149	68	M	?	?	mitral	–	–	carcinoma of prostate	–
22-229	23	F	?	?	mitral	+	–	peritonitis	–
23-252	31	F	1 wk.	?	mitral, aortic	–	?	scarlet fever	?
24-212	26	M	?	–	mitral	+	–	multiple sclerosis	embolic
24-566	26	M	?	?	mitral, aortic, tricuspid	–	–	thrombosis of cavernous sinus	–
24-745	12	M	?	?	mitral	–	–	acute osteomyelitis	–
25-103	68	M	?	?	mitral	+	–	infected wound	–

valves, nearly always shows a diffuse inflammation. Rarely this is of an acute proliferative character (Figs. 9 and 10); but more frequently it is of a more chronic type with large numbers of small fibroblasts, prominent blood vessels and some lymphocytes (Fig. 12). The inflammation may extend through the distal half of the valve (Fig. 1) or even to its attached margin. A section through the center of the opaque part of the leaflet shown in Fig. 1 shows a lymphocytic infiltration with many small fibroblasts. It is easy to understand how this lesion may give rise to a thickened scarred valve in the course of healing.

The structure of the vegetations in the recurrent cases is identical with that of the acute rheumatic lesions except that in general the lesions on defective valves are less active. There are usually fewer lymphocytes, smaller fibroblasts and more collagenous fibers. Healing processes are more in evidence and healed or partially healed vegetations are found along with the active ones. The valve underlying the vegetation is composed largely of scar tissue (Fig. 13).

Healing of Rheumatic Lesions. Relatively few persons die during the acute stage of rheumatic endocarditis. Death is usually due either to deformities of the valves that occur in the course of healing or to the subsequent development of a bacterial endocarditis on the diseased leaflets. Evidences of healing were found in all the recurrent and in two of the acute cases (24–364 and 24–1a) of rheumatic endocarditis. In the vegetations the first change noted is the formation of many new collagenous fibers along with a decrease in the size of the fibroblasts. These cells soon recede to the size of fixed tissue cells, and in the denser parts of the scar they usually disappear entirely. The central part of the vegetation becomes a scar-like structure. The lymphocytes emigrate or disintegrate. The peripheral hyaline layer becomes more homogeneous and glassy (Figs. 14 and 15) and remains in this condition indefinitely. It is not absorbed and does not become organized. Hyalin in the deeper parts of the vegetation or in the body of the leaflet may likewise persist indefinitely or it may become calcified. Within the hyaline masses there are very few fibroblasts and these disintegrate without forming collagenous fibers (Fig. 15). The end result of healing of a rheumatic vegetation is scar tissue sometimes with a thin hyaline layer on its surface. In the body of the leaflet healing results in an

increased amount of fibrous tissue which commonly assumes the appearance of a scar. This topic will be discussed further in connection with old valvular defects.

II. Bacterial Endocarditis[3]

This group includes all cases of active endocarditis except the rheumatic and the syphilitic. The vegetations are larger and softer than the rheumatic type. Their consistence is such that portions may become detached to form emboli. Bacteria are commonly present in the vegetations and in the circulating blood, but they cannot be demonstrated in all cases. There are no fundamental distinctions from rheumatic endocarditis, and lesions of the rheumatic type are present in a large percentage of the hearts of this group; but all hearts in which some of the vegetations are large and soft have arbitrarily been classified as bacterial.

1. Acute Bacterial Endocarditis. On clinical grounds it is convenient to classify all cases of less than six weeks' duration as acute and those of longer continuance as subacute. The acute cases may be further subdivided into those in which endocarditis is the only prominent clinical finding (*primary*) and those in which endocarditis is definitely overshadowed by some major illness which causes death (*secondary*).

a. In the *primary* group (Table 3) there are thirteen cases. The clinical picture is that of severe septicemia with or without physical signs of endocarditis. One death was due to cerebral embolism. Associated infections, presumably secondary to the septicemia, were found in four cases, *viz.*, bronchopneumonia, meningitis and pericarditis.

The vegetations are usually large and soft, but some small firm lesions of rheumatic type were occasionally seen. Ulceration of the leaflets was present in only one instance. In ten of the thirteen hearts only one of the valves was involved.

Only four hearts were available for microscopic study of the valves. Two of these showed necrosis of the marginal part of the leaflet with large platelet thrombi, and an exudate of polymorphonuclears and lymphocytes within the valve. In both of these, areas of inflammation of rheumatic type were found on the inner surface of the leaflet midway between the free and attached margins.

TABLE 5

Subacute bacterial endocarditis

Number	Age, in years	Sex	Duration acute symptoms	Valves involved	Old valvular defect	Arthritis, time before death	Weight of heart, in grams	Aschoff bodies	Pericarditis	Duration chronic valvular disease	Rheumatic lesions	Glomerulonephritis
10–73	42	F	3 mos.	mitral	–	several attacks	393	–	–	. .	–	?
10–76	25	M	3 mos.	mitral	–	?	normal	–	–	. .	. .	–
10–164	36	M	?	aortic	aortic, I_3	–	530	–	–	?	. .	–
12–76	46	M	10 wks.	mitral	–	continuous during attack	310	–	–	. .	. .	–
12–131	33	M	3 mos.	mitral, aortic	mitral, S. I. aortic, S. I.	–	460	–	–	?	+	subacute diffuse
13–100	39	M	?	aortic	aortic, I_2	?	675	–	–	?	. .	embolic and mild acute diffuse
13–165	33	M	4 mos.	mitral, aortic	aortic, I_3	a few years	640	–	–	o	+	acute diffuse
13–180	63	M	?	mitral	mitral, S_3	?	normal	–	acute	2 yrs.	–	mild acute diffuse
13–189	48	M	3 mos.	mitral	mitral, I_2	–	675	–	–	o	. .	embolic
13–190	35	F	6 mos.	mitral	–	20 yrs., 13 yrs., many other attacks	525	–	old	. .	+	acute diffuse
14–49	25	M	3 mos.	aortic, mitral	aortic, I_3, mitral, S. I.	several attacks	575	–	–	o	–	acute diffuse

14–207	43	M	6 mos.	aortic	aortic, I_3	one	620	–	–	o	+	–
15–30	55	M	3 mos.	aortic, mitral	aortic, I_2, mitral, S. I.	?	475	–	–	o	+	–
15–81	35	M	6 mos.	mitral	mitral	–	575	–	–	o	..	–
15–97	29	F	9 wks.	mitral	–	?	normal	–	–	..	+	acute diffuse
15–176	42	M	6 mos.	aortic, mitral	aortic, S_3, mitral, I_1	–	480	–	–	o	..	–
15–209	35	F	?	mitral, aortic	mitral, I. S., aortic, S. I.	?	425	–	–	?	..	mild acute diffuse
15–312	24	M	9 mos.	aortic, mitral	aortic, S. I., mitral, I_1	9 mos.	723	–	–	o	+	embolic
15–393	59	M	6 mos.+	mitral, aortic	–	?	normal	–	–	..	+	–
16–124	45	M	5 mos.	mitral	–	?	400	–	acute	..	+	embolic
16–152	6	F	3 mos.	mitral	–	–	243	–	acute and old	..	–	acute diffuse
16–203	61	M	2 mos.	aortic	aortic, S. I.	?	680	–	–	?	–	?
16–251	33	M	10 wks.+	mitral, tricuspid	mitral, S_3	21 mos.	574	–	–	15 mos.	..	embolic
16–295	11	F	2 mos.+	mitral, tricuspid	mitral, I_1	2 yrs. (chorea)	250	+	–	2 yrs.	+	embolic

TABLE 5 — *continued*

Number	Age, in years	Sex	Duration acute symptoms	Valves involved	Old valvular defect	Arthritis, time before death	Weight of heart, in grams	Aschoff bodies	Pericarditis	Duration chronic valvular disease	Rheumatic lesions	Glomerulonephritis
16–413	38	M	3 mos.	aortic, mitral	aortic, I. S., mitral, I_1	5 yrs.	860	–	–	?	+	embolic
17–28	29	M	11 mos.	aortic, mitral, tricuspid	aortic, I_2, mitral, I_2	11 yrs., 6 yrs.	690	+	acute	0	+	embolic
17–36	29	F	6 mos.	mitral	mitral, I_1	9 yrs., many since	250	–	–	14 yrs.	..	embolic, mild acute diffuse
17–174	28	M	7 mos.	mitral	mitral, I. S.	10 yrs.	565	+	–	4 yrs.	..	–
17–202	25	M	8 mos.	mitral	mitral, I_3	10 yrs., 9 yrs.	640	–	old	6 yrs.	..	acute diffuse
17–260	25	M	7 mos.	aortic, mitral	aortic, I_2 mitral, S_2	11 yrs., 7 mos.	570	–	–	11 yrs.	–	embolic, severe
18–102	23	M	4 mos.	aortic, mitral	aortic, I_3	?	690	–	–	0	+	–
18–105	13	F	11 mos.	mitral	–	–	350	–	–	..	–	–
18–122	30	M	3½ mos.	mitral, aortic	–	–	565	–	–	..	..	acute diffuse
18–175	34	M	8 mos.	aortic, mitral	–	?	480	–	–	..	..	embolic, severe
18–178	24	F	?	mitral, aortic	mitral, S_3	?	410	–	–	?	..	?
19–23	31	F	7 wks.	aortic	–	–	300	–	–	..	–	glomerular hemorrhages

19–141	55	M	6 wks.	aortic	–	–	325	–	–	..	+	–
19–161	23	F	8 mos.	mitral, tricuspid	–	–	365	–	acute	..	+	embolic, severe
19–264	29	M	?	mitral	–	2 yrs., 1 yr.	435	–	–	..	+	chronic diffuse
19–273	66	M	2 mos.	aortic	–	–	480	–	–	..	+	–
19–276	16	M	?	aortic, tricuspid	aortic, I_3	?	620	–	–	2 yrs.	–	acute diffuse
20–88	51	M	6 wks.	aortic, tricuspid	–	?	314	–	–	..	..	–
20–122	63	M	?	aortic	–	?	450	–	acute	..	–	–
20–165	43	M	6 mos.	aortic, mitral	–	–	625	–	–	..	+	–
20–296	16	M	7 wks.	mitral	–	–	330	+	–	..	..	–
20–326	28	M	2 mos.	mitral, aortic	mitral, I_1, aortic, I_2	12 yrs., 8 yrs., 5 yrs., 2 yrs.	920	–	old	several yrs.	+	?
20–344	12	F	5½ mos.	mitral, aortic	–	5½ mos.	280	–	–	..	+	embolic, severe
20–368	18	M	6½ mos.	mitral	–	?	340	–	acute	..	+	embolic and acute diffuse
21–45	55	M	4 mos.	mitral, aortic	–	–	420	+	–	..	+	embolic, severe
21–65	52	M	?	mitral	–	?	400	–	–	..	+	embolic
21–283	24	M	7 mos.	mitral	–	–	400	–	–	..	..	embolic
21–414	50	M	2 mos.+	mitral	–	35 yrs.	enlarged	–	old	..	–	–

TABLE 5 — *continued*

Number	Age, in years	Sex	Duration acute symptoms	Valves involved	Old valvular defect	Arthritis, time before death	Weight of heart, in grams	Aschoff bodies	Pericarditis	Duration chronic valvular disease	Rheumatic lesions	Glomerulonephritis
21–468	36	M	14 wks.	mitral	–	$3\frac{1}{2}$ mos.	450	–	–	. .	. .	?
21–513	42	M	10 wks.	aortic, mitral	–	8 yrs.	545	–	–	. .	+	?
21–559	28	M	14 wks.	mitral, aortic	mitral, I_2	–	490	–	–	0	. .	–
22–217	55	M	6 wks.	tricuspid	–	–	245	–	–	. .	–	–
22–287	50	M	?	aortic, mitral	aortic, I. S., mitral, I_1	4 yrs., 8 mos.	660	–	–	4 yrs.	+	–
22–418	41	M	?	aortic	–	?	675	–	–	. .	–	–
22–465	21	M	?	aortic	aortic, I_2	?	450	–	–	3 yrs.+	. .	?
22–554	43	M	5 mos.	aortic, mitral	–	?	610	–	acute	. .	+	chronic diffuse
23–390	45	M	?	aortic	–	–	575	–	–	. .	+	acute diffuse
23–484	46	M	$3\frac{1}{2}$ mos.	aortic, mitral	aortic, S. I.	9 yrs.	725	–	acute	9 yrs.	+	–
23–508	76	M	7 wks.+	aortic, mitral	aortic, S. I.	several attacks past few yrs., 12 wks.	550	–	–	?	–	embolic
24–4	30	M	5 mos.	tricuspid	–	–	300	–	–	. .	. .	embolic
24–91	39	M	11 mos.	aortic	aortic, I. S.	20 yrs., 11 mos.	580	–	–	10 yrs.	+	embolic

24-121	59	M	7 mos.	aortic, mitral	–	–	560	–	old	..	+	–
24-241	23	F	11 wks.	aortic, mitral	–	7 yrs., 11 wks. to present	330	–	old	..	+	embolic
24-605	35	M	3½ mos.	aortic	–	–	550	–	–	..	+	embolic
24-769	14	F	5 mos.+	mitral	mitral, I_1	chorea, 3 yrs., 7 mos., 5 mos.	255	+	–	3 yrs.	+	embolic, severe
24-770	10	F	4 mos.	aortic	–	4 mos.	380	–	acute	..	+	?
24-807	20	F	6½ mos.	mitral, tricuspid	–	–	400	–	–	..	–	acute diffuse
25-142	4	F	6 wks.	mitral	–	has chorea	125	–	–	..	..	–
25-362	42	M	6 wks.	pulmonary	–	–	420	–	acute	..	..	–
25-589	30	F	3 mos.	mitral, aortic	mitral, I. S., aortic, I_2	8 attacks past 12 yrs., present now	350	–	old	?	+	acute diffuse
11-122	51	M	6 wks.	mitral, aortic	mitral, S_3	16 yrs., 1 yr., several between	575	–	–	16 yrs.	+	–

Legend: S = stenosis. I = insufficiency. S. I. = chiefly stenosis. I. S. = chiefly insufficiency. The degree of stenosis or insufficiency is indicated by the subnumbers 1, 2, 3.

In the two others there were large fresh soft thrombi with an extensive exudative reaction within the leaflet. The leucocytes were chiefly polymorphonuclears. There was some proliferation. Numerous bacteria were found in the thrombotic material of one of these.

b. Twenty-seven cases of *secondary acute bacterial endocarditis* (Table 4) were studied. The clinical symptoms were always those of the major illness. The endocarditis was not recognized clinically in any case, but perhaps it might have been diagnosed in some had more attention been given to the heart. The heart lesion was not considered by the pathologist as the main cause of death in any instance. In this group are seven cases of puerperal sepsis following induced abortion. The inclusion of this disease presumably explains the preponderance of females.

In twenty cases there was clinical evidence of septicemia which was attributable to the major illness, but in the remaining seven the usual signs of infection were absent.

The mitral valve was affected twenty-five times, the aortic five times and the tricuspid once. More than one valve was involved in only four cases. In only one heart was the acute lesion found on a previously thickened valve. The vegetations cannot be distinguished grossly from those of subacute bacterial endocarditis. In general, however, they are fewer in number. Ulceration of a leaflet was found only once.

Six hearts of this group were available for microscopic study of the valves. Four showed a large soft thrombus with necrosis of the leaflet and a purulent exudate within the valve. In one of these the single vegetation was 1 cm. in diameter. Bacteria were very prominent in another.

In the fifth and sixth cases there was very little exudate, the lesions being of proliferative type with prominent evidences of healing.

The lesions of acute bacterial endocarditis do not differ in any essential respects grossly or microscopically from those of the subacute bacterial type. Any distinction that is made must therefore be based chiefly on clinical data.

2. Subacute Bacterial Endocarditis. Seventy-four cases of this group have been studied (Table 5). The literature on this form of endocarditis has previously been reviewed by one of us.[4] These cases correspond clinically with subacute bacterial endocarditis as defined by Libman.[5]

The duration of symptoms in our series was as follows: 6 weeks to 3 months, 27; 3 to 4 months, 9; 4 to 5 months, 2; 5 to 6 months, 9; 6 to 7 months, 7; 7 to 8 months, 3; 8 to 9 months, 1; 10 to 11 months, 3; indefinite duration, 13. All the patients died within one year of the first appearance of symptoms.

Thirty-one of the seventy-four patients gave a history of one or more attacks of *acute arthritis*, and in eleven of these the fatal illness began with such an attack. A valvular lesion associated with acute arthritis may therefore be of the subacute bacterial type. The number of attacks varied from one to eight, and some occurred as long as twenty years before death. Twenty-four patients had never had arthritis, and in the nineteen others the history was indefinite on this point.

Relation to Previous Valvular Disease. Twenty-three patients gave a definite history of chronic valvular disease. In fifteen the duration was from one to sixteen years, and in eight the exact duration was unknown. Fifteen of these twenty-three patients gave a history of acute rheumatic fever which apparently caused the valvular defect.

In thirty-four instances the leaflets were grossly thickened and scarred, indicating a previous inflammation; but, since a considerable amount of scar tissue may form during the course of a subacute case, only extreme thickening can be considered positive anatomic evidence of a previous old valve defect. In forty cases there was no clinical evidence of old valve defect and the valves were not notably thickened. Apparently over half the subacute cases begin on previously normal valves. It is to be noted that when old defective valves are present in these hearts they always show an active inflammation. Apparently they are more susceptible to infection than normal leaflets.

In several instances the endocarditis appeared to develop secondarily to some other infectious process, *viz.*, otitis media (2 cases), lupus erythematosus (1), empyema (1), tonsillitis (1).

In the 74 hearts the mitral was diseased in 56, the aortic in 44, the tricuspid in 9 and the pulmonary in 1. A single valve was affected in 38 instances; mitral 22, aortic 13, tricuspid 2, pulmonary 1. The most frequent combination was mitral and aortic, 29 hearts.

Gross Appearance of Valves. The diagnostic feature is the large soft vegetation, but the appearance of the valves varies widely with the

number and size of the vegetations, and the extent of ulceration, thickening and calcification. Ulceration with loss of substance of the leaflet was present in thirty-two hearts. The term "ulcerative" endocarditis can properly be applied only to this number. The ulcers vary from a diameter of a few millimeters to complete destruction of a leaflet. They are more frequently found on the aortic valve. In three cases there was perforation of a leaflet, and in two there were ulcers penetrating to the right auricle from the aortic area.

Varying degrees of calcification of the valves were noted in twenty-five hearts. Calcium is deposited in the thrombotic material and in the old hyaline scar tissue. In the former position it is commonly present in moderate amounts, such as to give a gritty feel when cut; in the latter position, however, it is often of bony density. Calcium is readily deposited in the larger thrombi independently of the stage of healing. Frequently it is found in definitely active vegetations and even in association with bacteria.

In addition to the larger vegetations, small firm vegetations of the rheumatic type can be seen grossly in about three-fourths of the hearts, if a careful search is made for them. Sometimes one valve shows only rheumatic vegetations, but usually both types are found on the same leaflet.

Situation of the Lesions in Active Endocarditis. (*a*) *Aortic valve.* In acute rheumatic endocarditis the vegetations were always found on the ventricular surface of the leaflet in a line about one-third the distance from the free to the attached margin. This corresponds to the line of contact of the leaflets when the valve is closed. In recurrent rheumatic endocarditis the situation of the vegetations was the same except in one case in which they were found only on the free margin of one leaflet. In twenty-one cases of the subacute bacterial type, the vegetations occupied the same position as in the rheumatic form, but in twelve other cases they extended from this line around the free edge onto the aortic surface. In one case they were found only on the free margin of the leaflet.

(*b*) *Mitral valve.* In the acute rheumatic type the line of vegetations was always on the auricular surface, 1 to 2 mm. from the free margin, with one exception in which the vegetations were larger and extended over the free margin onto the chordae. In the recurrent type the vegetations occupied the same position as in the

acute type with two exceptions, in one of which they were on the free margin only and in the other on the ventricular surface only, near the free margin. In eighteen cases of the subacute bacterial type the vegetations extended from a line 1 to 2 mm. back of the free margin for varying distances upon the auricular surface. In thirteen other cases they extended from the auricular surface around the free margin onto the ventricular surface. In five hearts the vegetations were found only on the ventricular surface of the aortic leaflet of the mitral, extending from the central part of its surface toward the free margin. In all five of these the adjacent aortic leaflet also showed similar vegetations. A vegetation on this aortic leaflet may strike against the central part of the ventricular surface of the aortic leaflet of the mitral.

(*c*) *Tricuspid valve.* Acute lesions of both types were always found on the auricular surfaces of the leaflets near the free margin.

(*d*) In the single instance in which the *pulmonary valve* was involved, the lesion was of bacterial type and affected the margins as well as both surfaces of the leaflets.

Microscopic Structure. In forty-six hearts the valves were studied microscopically, one or two areas from each valve. Both exudative and proliferative forms of inflammation are usually found in the sections examined from each heart; but frequently one type of reaction predominates. Both types may be found in the same leaflet. The proliferative reaction is most common.

The exudative lesions are similar to acute exudative inflammation in other tissues. Rarely they are definitely purulent in character. The leucocytes are either polymorphonuclears or mononuclears. In one valve a number of embolic abscesses were found (Fig. 16). The thrombus overlying an exudative lesion is fresh and soft.

Proliferative inflammation is seen more frequently than the exudative. In the valve underneath the thrombus are large numbers of fibroblasts with relatively few leucocytes. The fibroblasts may be small and separated by a considerable number of collagenous fibers (Fig. 17); or they may be large with only a minimum of intercellular fibers (Fig. 18). In slowly progressive or healing lesions the fibroblasts tend to be smaller and the intercellular collagenous fibers more prominent. In sixteen hearts the proliferative lesions were prominent and the exudative inconspicuous. In five hearts large

multinucleated fibroblasts, such as occur in Aschoff bodies, were found (*cf.* Fig. 18). The histologic structure of the lesions in the valves seems to have no direct relation to the clinical course of the disease.

Necrosis of part of a leaflet is frequently seen. Ulceration is a direct result of necrosis.

In addition to the bacterial lesions just described, which predominate in these hearts, lesions of the rheumatic type were found in thirty-five of the forty-six that were examined microscopically. These vegetations have the same structure as those found in typical rheumatic endocarditis. In four hearts the tricuspid valve showed typical gross rheumatic vegetations but none of the bacterial type. Usually both types are found on the same valve. The rheumatic lesions may be continuous with the bacterial. The rheumatic vegetations were active in 22 hearts, healing in 9, and both active and healing in 4. In the 35 cases with rheumatic lesions 18 gave a positive history of rheumatic fever, 9 a negative history, and in 7 the history was incomplete. In the 31 cases with positive history of rheumatic fever, rheumatic lesions were present in 18 and absent in 5 (8 not examined). In 11 cases that began with acute arthritis, rheumatic lesions were present in 7 and absent in 2 (2 not examined). It cannot be said that the rheumatic vegetation is pathognomonic of rheumatic fever since it is found in three-fourths of subacute bacterial cases.

Evidences of healing are found in a great majority of these valves, and often the signs of active inflammation have largely disappeared. There may be active inflammation in one part of a leaflet and advanced healing in another part. In the leaflet itself the fibroblasts decrease in size while many new collagenous fibers are being formed. The leucocytes emigrate or disappear. The final result is dense scar tissue underlying a hyaline thrombus (Fig. 19).

The thrombus may soften and disintegrate, especially when it contains a large proportion of leucocytes. Portions may become detached to form emboli. Those portions that remain permanently attached to the leaflet soon become homogeneous in structure. They may persist indefinitely in this condition, or they may become calcified. Calcification may give rise to hard nodular masses or to diffuse hardening. Organization is rarely seen, and seems never of sufficient extent to convert a thrombus into scar tissue.

The most serious valvular defects result from necrosis and sloughing, but some deformities are due to the contraction of scar tissue. The importance of bacterial endocarditis in the production of old valvular defects will be discussed in a subsequent paragraph.

III. Old Valvular Defects [6]

This group includes cases of chronic valvular disease in which there were no clinical indications of active endocarditis, and in which the valves showed gross thickening and scarring. In 114 of the 130 cases of this group death was due to cardiac failure. In the 16 remaining cases the immediate cause of death was as follows: coronary sclerosis, 2; primary hypertension, 2; intestinal obstruction, 1; pyemia, 1; tuberculosis, 1; Addison's disease, 1; peritonitis, 1; suicide, 1; hemorrhage from gastric ulcer, 1; luetic aortitis, 1; cirrhosis of liver, 1; embolism of the stenosed mitral orifice, 1; and undetermined, 2.

The mitral valve was diseased in 95, the aortic in 82, the tricuspid in 13, the pulmonary in 3. The mitral alone was involved in 44, the aortic alone in 32, the tricuspid alone in none, the pulmonary alone in 3. The aortic and mitral were both diseased in 50 cases. The aortic, mitral and tricuspid valves were all three involved in 12 cases.

There were thirty-seven cases of pure mitral stenosis (*i.e.*, extreme stenosis with negligible insufficiency), and twelve cases with varying degrees of mitral insufficiency in which stenosis was negligible. In forty-three other hearts the mitral was both stenosed and insufficient, the stenosis dominating in thirty-five, the insufficiency in eight. In three hearts in which the mitral only was diseased, the lesion was not severe enough to cause any functional disturbance. In nineteen hearts an adherent pericardium was found, and in some of these it was the chief cause of the cardiac failure.

There were twenty cases of pure aortic stenosis, and seventeen with varying degrees of insufficiency without stenosis. In forty-one hearts the aortic valve was both stenosed and insufficient, the stenosis dominating in twenty-eight, the insufficiency in thirteen. In four hearts the lesion caused no functional disturbance. Aortic stenosis is not a rare type of chronic valvular disease.

The tricuspid lesions were usually of mild degree. The three pulmonary lesions were pure stenosis of congenital type.

Patients with mitral stenosis died at an earlier average age than those with aortic stenosis. In forty-three cases in which the mitral lesion was chiefly or entirely stenosis with a normal aortic valve, the average age at death was 41.7 years. In twenty-nine cases in which the aortic lesion was chiefly or entirely stenosis, with a normal mitral valve, the average age at death was 55.8 years.

The duration of symptoms of chronic valvular disease of all types was as follows: less than 1 day, 5 cases; 1 day to 3 months, 18; 3 to 6 months, 8; 6 to 12 months, 12; 1 to 2 years, 15; 2 to 3 years, 9; 3 to 4 years, 11; 4 to 5 years, 4; 5 to 10 years, 13; 10 to 20 years, 9; 20 to 34 years, 3; indefinite number of years, 4; and unknown duration, 14. With respect to the duration of symptoms there are no important differences between mitral and aortic stenosis.

There was a positive history of one or more attacks of rheumatic fever in fifty of the 130 patients, and a negative history in thirty. In fifty cases there was no mention of rheumatic fever. In one negative case the symptoms began after an attack of tonsillitis.

In sixteen cases of pure mitral stenosis without pericardial adhesions and without involvement of other valves, the average weight of the heart was 441 gm.; minimum weight 270 gm.; maximum 680 gm. The enlargement is chiefly right ventricular hypertrophy.

In thirteen cases of pure aortic stenosis without pericardial adhesions and without involvement of other valves, the average weight of the heart was 705 gm.; minimum 475 gm.; maximum 1130 gm.

The average weight of the heart in all the cases of chronic valvular disease was 580 gm.

Adherent pericardium (old adhesions) was found in nineteen cases, and acute pericarditis in six.

The most frequent gross change in the valves is thickening and stiffening with resulting loss of elasticity and narrowing of the orifice; but often there is retraction and curling with insufficiency which may be more pronounced than stenosis. The leaflets of the aortic valve are often fused together at the aortic attachment where they come into contact.

Calcification is a very common change in old defective valves, being present in varying degree in eighty-five of the 130 cases. The calcium is either distributed diffusely or in the form of large nodular

masses. Diffuse calcification was found in the mitral in forty-one instances and in the aortic in thirty. The calcium is deposited in the hyaline scar tissue within the leaflet.

Seventy-three of the 130 hearts of this group had been preserved and were available for careful gross and microscopic study. The remaining fifty-seven were described in the necropsy protocols, but no material except pieces of ventricular muscle was kept. Tables 6, 7 and 8 were made from the seventy-three preserved specimens. These hearts have been arranged in three groups for convenience of description: Group 1, in which the thickened valves are incompletely healed; Group 2, in which the valves are thickened and stiffened from newly formed fibrous tissue but show no unhealed areas; Group 3, in which the thickening of the leaflets is due chiefly to large calcareous nodules.

Group 1. Incompletely Healed Valves
30 Cases (Table 6)

The unhealed areas can usually be seen with the unaided eye, but often they are more readily recognized with a small hand lens. In eleven hearts they appeared as small firm pale vegetations, usually in the form of a slightly elevated ridge but sometimes very conspicuous (Fig. 20). Some of these valves are not distinguishable grossly from the recurrent rheumatic group, but usually the lesions are less prominent. In three hearts there were large ulcerated areas covered by a thin layer of thrombus (Fig. 22). In the remaining sixteen hearts the lesions appeared as small roughened denuded areas (Fig. 24).

Microscopic Structure. The thickened valves of this group are composed largely of dense fibrous tissue, often hyaline in structure, such as results from the healing of inflammatory processes in any tissue. But sections cut through the unhealed areas show a recognizable rheumatic or bacterial lesion in some stage of healing. It may be a well defined elevated vegetation, easily recognized as rheumatic. The one shown in Fig. 25 has a core composed of dense fibrous tissue in which capillaries are still present, and the surface hyaline layer is conspicuous. The one shown in Fig. 26 is of dense hyaline structure, but the surface hyaline layer may still be recognized on one side. A microscopic section of the vegetations seen in

TABLE 6

Old defective valves. Group 1. Incomplete healing

Number	Age, in years	Sex	Duration	Acute arthritis, time before death	Valves				Weight of heart in grams	Old pericardial adhesions	Calcareous nodules	Incomplete healing	Aschoff bodies
					mitral	aortic	tricuspid	pulmonary					
10–9	45	M	6 yrs.	20 yrs.	S. I.	S_3	I_1	–	540	–	–	mitral, raw area	–
10–142	47	M	?	?	S. I.	–	–	–	250	–	–	raw area	–
11–121	34	M	1 yr.	6 yrs., 3 yrs.	I_2	–	–	–	350	–	–	vegetations	–
12–117	54	M	16 mos.	?	I. S.	–	–	–	400	–	–	thrombus on large ulcer	–
12–168	27	M	4 yrs.	12 yrs., 8 yrs., 2 yrs.	S_3	S. I.	–	–	400	+	–	mitral, raw area	–
14–20	20	M	2 yrs.	?	S_3	I_1	o	–	370	–	–	tricuspid, vegetations; aortic, raw area	–
15–323	49	M	?	several attacks	S_3	–	–	–	325	–	–	thrombus on large ulcer	–
15–324	53	M	3 yrs.	28 yrs.	S. I.	I. S.	–	–	795	–	–	mitral, aortic, vegetations	–
16–16	29	M	27 mos.	10 yrs.	S_3	–	–	–	350	–	–	raw area	–
16–185	46	M	?	?	S. I.	–	–	–	320	–	–	thrombus on large ulcer	–
19–26	45	M	3 yrs.	?	S_3	–	–	–	510	–	–	raw area	–
19–286	51	M	1 yr.	?	S_3	–	–	–	550	–	–	raw area	?
20–157	61	M	3½ yrs.	?	S_3	S. I.	S_1	–	550	–	–	mitral, raw area	+
20–250	65	M	4 yrs.	?	o	S_3	–	–	715	–	aortic, mitral	aortic, raw area	–

20–358	21	F	12 hrs.	?	S_3	o	–	–	270	–	–	aortic, vegetations	–
20–433	57	M	5 mos.	40 yrs.	o	S_3	–	–	720	–	aortic, mitral	raw area, one leaflet	–
20–463	37	M	3 yrs.	19 yrs., 13 yrs., 4 yrs., 3 yrs.	I. S.	I. S.	–	–	825	–	aortic	mitral, aortic, raw area	–
21–259	52	F	2 wks.	–	S. I.	–	o	–	325	–	–	tricuspid, vegetations	–
21–285	53	M	10 yrs.	20 yrs.	–	I_2	–	–	475	–	aortic	raw area	–
21–416	46	M	6 wks.	20 yrs.	I. S.	I. S.	–	–	900	+	–	mitral, vegetations	–
21–473	58	F	7 mos.	?	S. I.	–	–	–	550	–	–	raw area	–
21–485	60	F	?	?	S_3	–	–	–	410	–	–	raw area	–
22–197	41	F	9 mos.	?	S_3	–	–	–	350	–	–	vegetations	–
23–142	35	M	5 yrs.	5 yrs.	–	S_3	–	–	850	–	–	raw area	–
23–324	42	M	3½ mos.	–	I. S.	S. I.	–	–	550	–	–	raw area	+
24–307	43	F	?	?	o	–	–	–	430	–	–	vegetations	–
24–482	40	M	?	?	S. I.	–	–	–	365	–	–	vegetations	–
24–613	38	F	?	?	o	–	–	–	375	–	–	vegetations	–
25–322	33	M	5 yrs.	?	S. I.	I.S.	–	–	690	–	–	aortic, mitral, raw areas	–
25–328	70	M	?	?	o	–	–	–	300	–	–	vegetations	–

Legend: S = stenosis. I = insufficiency. S. I. = chiefly stenosis. I. S. = chiefly insufficiency. The degree of stenosis or insufficiency is indicated by the subnumbers 1, 2, 3.

Fig. 20 is shown in Fig. 21. It consists of a rather thick layer of old hyaline thrombus resting on dense scar tissue. It suggests a healed bacterial type of lesion because of the thickness of the thrombus.

The raw areas show a variety of microscopic appearances. Occasionally fibroblasts are to be seen and there are other signs of low grade activity. Usually there are no signs of active inflammation. A layer of hyaline thrombus rests upon dense fibrous tissue (Fig. 27) or upon hyaline scar tissue (Fig. 28).

In one instance (25–322) the leaflet underlying the raw area showed areas of necrosis and numerous polymorphonuclear leucocytes as well as dense fibrous tissue. The ulcer shown in Fig. 22 likewise shows an active exudative inflammation within the substance of the valve (Fig. 23). Neither of these patients had any clinical signs of active endocarditis. They died apparently from the valvular defect and not from toxemia. The two hearts shown in Figs. 20, 21, 22 and 23 as well as one other are interpreted by us as old valvular defects resulting from subacute bacterial endocarditis. The other hearts of this group are considered to have developed from the rheumatic type of endocarditis.

There was a history of rheumatic fever in eleven of this group of thirty patients. The mitral valve was involved alone in 18; the aortic alone in 4; mitral and aortic, 6; mitral, aortic and tricuspid, 3. In four hearts there were calcareous aortic nodules characteristic of Group 3. In seventeen hearts one or more valves were partly calcified.

Group 2. Thickened Valves Composed of Dense Fibrous Tissue; No Unhealed Areas

28 Cases (Table 7)

In this group the valves have the same gross appearance and microscopic structure as those of Group 1 except that there are no areas of incomplete healing. All signs of active inflammation are absent except occasionally a little perivascular lymphocytic infiltration in the central part of the leaflets. The leaflets are composed of dense fibrous tissue which is often hyaline in appearance. At the surface one frequently sees a thin hyaline layer that suggests the surface hyaline layer of a rheumatic vegetation (Fig. 29). The valves of this group usually show areas that correspond in structure

to the undoubted healed rheumatic lesions found in recurrent rheumatic endocarditis (Fig. 15). The histologic structure, therefore, offers strong support for the interpretation of these valves as healed rheumatic endocarditis. The clinical and gross pathologic features parallel the cases of Group 1 in which this interpretation seems amply justified.

There was a history of acute arthritis in thirteen of the twenty-nine cases of this group. The mitral valve was involved alone in 12; the aortic alone in 2; mitral and aortic in 10; mitral, aortic and tricuspid in 5. The calcareous aortic nodules characteristic of Group 3 were found in two hearts.

Group 3. Cases of Aortic Stenosis Due to Calcareous Nodules; No Satisfactory Evidence of Inflammatory Origin

15 Hearts (Table 8)

In the fifteen hearts of this group, ten were pure stenosis and in the other five stenosis predominated but there was some insufficiency. There is a very marked thickening and stiffening of the leaflets due to large calcareous nodules within them (Fig. 30). The portions of the leaflets between the nodules are of normal thickness. There is usually fusion of the adjacent edges of the leaflets where they are attached to the aorta. The nodules are found on the aortic surfaces of the leaflets but when very large they cause projections on the ventricular surfaces also. The most frequent site is near the aortic attachment but they usually extend well out into the leaflet, frequently to its free margin. In one instance a row of nodules extended about 2 cm. up the root of the aorta. In twelve of the fifteen hearts there were similar nodules in the central part of the ventricular surface of the aortic leaflet of the mitral and frequently there was a continuous row of nodules from the aortic leaflet to those on the mitral.

The root of the aorta is almost invariably free of arteriosclerotic lesions. The nodules are of whitish color, never yellowish. There seems to be no relation between these nodules and the atheromatous lesions so frequently seen on the valves.

The surface endothelium is usually intact over the nodules but occasionally it is denuded so that the calcareous material is ex-

TABLE 7

Old defective valves. Group 2. Complete healing

Number	Age, in years	Sex	Duration	Acute arthritis, time before death	Valves				Weight of heart, in grams	Old pericardial adhesions	Calcareous nodules	Aschoff bodies
					mitral	aortic	tricuspid	pulmonary				
16-212	27	M	14 yrs.	14 yrs., several attacks since	S_3	I. S.	–	–	700	+	–	+
17-139	33	F	15 yrs.	–	S. I.	–	–	–	525	–	–	–
18-67	22	M	7 mos.	10 yrs., 5 yrs.	S_3	S. I.	–	–	750	–	–	–
18-124	32	F	18 mos.	12 yrs., 9 yrs.	I_2	–	–	–	675	+	–	?
19-58	39	M	4 yrs.	–	S. I.	I_1	–	–	585	–	–	–
19-120	43	F	6 mos.	29 yrs., 16 yrs., 3 yrs.	S_3	S. I.	S. I.	–	600	–	–	–
19-145	16	F	7 yrs.	–	S. I.	S. I.	S. I.	–	400	–	–	–
20-452	32	M	3 yrs.	5 yrs., 4 yrs.	S_3	I. S.	I_1	–	500	+	–	–
20-459	40	F	8 yrs.	–	S. I.	I_1	I_1	–	410	–	–	–
21-205	22	F	?	?	0	I_2	–	–	490	–	–	+
21-478	38	M	2 mos.	3 yrs.	S. I.	S. I.	–	–	400	–	–	–
22-147	47	M	several years	?	S_3	S.I.	–	–	600	–	aortic, mitral	–
22-300	28	M	6 yrs.	?	S. I.	–	–	–	550	–	–	–
22-309	39	M	6 yrs.	–	S_3	–	–	–	510	–	–	–

22–354	68	M	2½ yrs.	–	I_3	o	–	–	645	+	–	–
22–365	51	M	10 yrs.	many attacks 25 yrs. to 3 yrs.	S_3	S_3	–	–	640	–	–	–
22–476	48	M	many years	?	S_3	–	–	–	550	–	–	–
22–480	27	M	5 yrs.	8 yrs, 5 yrs.	S_3	I_1	S. I.	–	550	–	–	–
23–101	43	M	3½ mos.	5 yrs.	I. S.	S. I.	–	–	550	+	–	+
23–135	48	F	5 mos.	date (?)	S_3	–	–	–	350	–	–	–
23–142	35	M	5 yrs.	5 yrs.	–	S_3	–	–	850	–	–	–
23–741	22	M	10 yrs.	–	–	I_3	–	–	1150	+	aortic	–
24–14	42	M	4 yrs.	22 yrs.	I. S.	I.S.	–	–	610	+	–	–
24–29	44	F	3 yrs.	28 yrs., 10 yrs.	S_3	–	–	–	500	–	–	–
25–389	45	F	2 yrs.	?	S_3	–	–	–	360	–	–	–
25–574	39	M	3 mos.	–	S. I.	–	–	–	600	–	–	–
25–655	21	F	2 yrs.	7 yrs., 6 yrs., 2 yrs.	I. S.	–	–	–	420	–	–	–
25–666	64	F	4 yrs.	?	I_1	I. S.	–	–	595	–	–	–

Legend: S = stenosis. I = insufficiency. S. I. = chiefly stenosis. I. S. = chiefly insufficiency. The degree of stenosis or insufficiency is indicated by the subnumbers 1, 2, 3.

posed. The position of the nodules in no way corresponds to the vegetations of active endocarditis. Mönckeberg[7] finds that they originate within the fibrous layer of the leaflets which is continuous with the wall of the aorta.

Microscopic Structure. The nodules consist chiefly of masses of calcium. When decalcified a homogeneous material remains. It has not been determined what kind of tissue calcifies. Surrounding the calcium there is usually some loose connective tissue, containing fat both within the cells and in the stroma, such as is found in atheromatous areas in the aorta. There are also areas of hyaline fibrous tissue. Often the adjacent tissue is very vascular and contains many mononuclear leucocytes, a reaction which may be interpreted as a change preliminary to the replacement of the calcium by bone. In one valve true cartilage and bone were found. A similar reaction is often seen in the calcified mitral leaflets of an ordinary mitral stenosis in which no calcified nodules are present.

Our material is not sufficient to enable us to trace the development of these aortic nodules. They were found in the aortic valves in twelve out of 100 adults dead of primary hypertension. Their incidence in other diseases has not been studied by us. Mönckeberg[7] believes that they begin as small calcified areas in the fibrous layer of the valve leaflet near the aortic attachment and gradually increase in size and number until an aortic stenosis may develop. He considers them entirely unrelated to inflammations of the valves.

These calcareous aortic nodules were found in four hearts of Group 1 and two hearts of Group 2 (see Tables 7 and 8), in fifteen out of fifty-four cases of subacute bacterial endocarditis and in three cases of recurrent rheumatic endocarditis. Whether they are the result of the inflammation in these valves or merely accidentally associated with it was not determined. It is to be noted that they were present in one person only 22 years old (23–741, Table 7).

Small calcified nodules are sometimes found in thickened leaflets at the site where vegetations occur. These seem not to be related to the aortic nodules just described, although they cannot be distinguished microscopically.

Calcareous nodules similar to those on the aortic valves are found occasionally on the ventricular surface of the marginal leaflet of the mitral at its attachment to the ventricle. These may occur independently of any other lesion in any valve.

TABLE 8

Old defective valves. Group 3. Calcified nodular type

Number	Sex	Age, in years	Duration	Acute arthritis, time before death	Valves				Weight of heart in grams	Old pericardial adhesions	Calcareous nodules	Aschoff bodies
					mitral	aortic	tricuspid	pulmonary				
15–38	M	66	9 wks.	1 yr.	o	S. I.	–	–	600	–	aortic, mitral	–
18–38	F	70	1 hr.	?	o	S. I.	–	–	425	–	aortic, mitral	–
18–143	M	51	10 yrs.	21 yrs.	–	S_3	–	–	600	–	aortic	–
18–167	M	50	1 yr.+	?	o	S_3	–	–	780	–	aortic, mitral	–
19–93	M	40	2 mos.	–	o	S_3	–	–	760	–	aortic, mitral	+
19–213	M	68	3 wks.	–	–	S_3	–	–	650	–	aortic	–
21–49	M	59	2 yrs.	?	o	S. I.	–	–	795	–	aortic, mitral	–
21–433	M	71	1 yr.	–	o	S_3	–	–	475	–	aortic, mitral	–
22–99	M	50	?	?	o	S_3	–	–	560	–	aortic, mitral	–
23–112	M	56	7 yrs.	?	o	S. I.	–	–	575	–	aortic, mitral	+
23–236	M	70	20 yrs.	?	o	S_3	–	–	500	–	aortic, mitral	–
23–320	M	44	?	?	o	S_3	–	–	1130	–	aortic, mitral	+
25–82	M	64	2 yrs.	?	–	S. I.	–	–	725	+	aortic	–
25–104	M	38	2 yrs.+	–	o	S_3	–	–	575	–	aortic, mitral	–
25–586	M	66	1 yr.+	?	o	S_3	–	–	500	+	aortic, mitral	–

Legend: S = stenosis. I = insufficiency. S. I. = chiefly stenosis. I.S. = chiefly insufficiency. The degree of stenosis or insufficiency is indicated by the subnumbers 1, 2, 3.

The average age of the twenty-one cases with aortic nodules is 54.5 years. Excluding the three youngest cases the average is 58 years. Apparently this type of valvular disease usually does not produce symptoms until late in life.

Nineteen of the seventy-three hearts with old valvular defects that are listed in Tables 6, 7 and 8 were examples of aortic stenosis (chiefly or entirely stenosis) without involvement of any other valve, and it is to be noted that in seventeen of these the aortic defect was due to calcareous nodules. Aortic stenosis in the absence of involvement of any other valve is therefore usually due to calcareous nodules.

Discussion

The initial stages of acute endocarditis are not definitely known. It has not been recognized in the absence of vegetations. There is a diffuse inflammation throughout the part of the leaflet adjacent to the free margin in the earliest stages, but it is not known whether this precedes the vegetations or *vice versa.* The study of serial sections from early cases, however, gives one a strong impression that the inflammation begins within the leaflet and extends to the surface, resulting in a vegetation. When only a few vegetations are present they are almost invariably situated on the inner surface along the line where the leaflets come into contact, but when there are many they often cover both surfaces of the leaflet, especially near its free margin. It may be inferred, therefore, that the trauma of contact has some causal relation to the first vegetations but not to those that form subsequently. It is now known that normal valves are supplied with blood vessels (Bayne-Jones,[8] L. Gross,[9] Kerr[10]) and that it is therefore anatomically possible for bacterial emboli to lodge within the valves as was maintained by Köster,[11] Rosenow[12] and others. We have no new observations as to the route of the primary infection except the finding of numerous bacterial emboli in one valve (Fig. 16).

In five hearts with aortic bacterial lesions the only mitral vegetations were situated on the central part of the outer surface of the aortic leaflet. In these instances the mitral lesion probably resulted from contact with an infected aortic leaflet.

To understand the structure of old defective valves it is very im-

portant to note that in the acute stages the vegetation is not the only lesion but that there is a diffuse inflammation of the leaflet, always in its distal part and often extending well toward its attached margin (Figs. 1 and 12). This is the reason why an old defective valve leaflet is commonly thickened throughout, with the maximum involvement near the free margin.

Rheumatic vegetations are largely proliferative in character. There are some lymphocytes and occasionally a few polymorphonuclears, but most of the cells are fibroblasts. There is practically no necrosis or ulceration and there is no organization since there is almost no material to be replaced. Lymphocytes are numerous in the valve apart from the vegetations. Healing occurs readily and always results in thickening of the leaflet, since the fibroblasts form many new collagenous fibers. As in any healing inflammation the newly formed fibrous tissue contracts, compressing the blood vessels and forming scar tissue. In time the scar tissue becomes hyaline in structure and it frequently becomes calcified. The thin layer of hyaline material on the surface of fresh rheumatic vegetations can often be recognized in old defective valves long after all signs of active inflammation have disappeared (Fig. 29).

In recurrent rheumatic endocarditis there is either an activation of a latent infection or a reinfection. Fresh vegetations form on valves already thickened by a previous inflammation and fresh leaflets become involved. Each attack leaves the valves thicker and more rigid than they were previously.

It is only a short step from the recurrent rheumatic to the old defective valve. The inflammation subsides and the clinical picture changes from infection to valvular defect. There is often a striking gross resemblance between these two stages (Fig. 20), though they may be distinguished by the clinical history and microscopic structure. Partially or completely healed vegetations may be found (Figs. 25 and 26), and in the raw areas the remnants of rheumatic inflammation are readily recognized (Fig. 28). Twenty-seven of the old defective valves of Group 1 show incompletely healed lesions recognizable as rheumatic. When the valves are completely healed (Group 2) the evidence of a rheumatic origin is not so obvious; but they are strikingly similar in structure to known healed rheumatic lesions (*e.g.*, those of recurrent rheumatic endocarditis, *cf.* Fig. 15 with Fig. 29), and they are parallel in clinical and gross pathologic

features to Group 1. Fifty-five of the seventy-three old defective valves are interpreted as of rheumatic origin.

Bacterial lesions differ from rheumatic only in the intensity of the inflammatory reaction. There are no sharp distinctions. Exudation is more abundant, the fibroblasts are more numerous, thrombi form on the injured areas and there is often necrosis and ulceration. Healing processes are found almost constantly but complete healing rarely occurs. The leaflet becomes fibrous and hyaline in certain parts. The thrombus soon becomes homogeneous. Portions may become detached. It may persist indefinitely as a hyaline thrombus or it may become calcified. We have found no evidence that organization occurs to any appreciable extent. Only three of the seventy-three old defective valves are interpreted by us as healed bacterial lesions, but even these were not clinical examples of bacterial endocarditis.

Rheumatic vegetations were found in three-fourths of the hearts of subacute bacterial endocarditis, and numerous transitions between the two types of vegetations were seen. Unless the improbable assumption is made that three-fourths of the subacute bacterial cases have a simultaneous acute rheumatic infection, it must be granted that rheumatic and subacute bacterial endocarditis are caused by the same organism.

Glomerulonephritis was found in 38 of 64 cases of subacute bacterial endocarditis. The type of glomerulonephritis was embolic in 19, acute diffuse in 13, subacute or chronic diffuse in 3, and a combination of embolic and acute diffuse in 3. Diffuse glomerulonephritis as a complication of subacute bacterial endocarditis is about as frequent as the embolic form. Baehr and Lande [13] called attention to this relationship in 1920.

Aschoff bodies were found in the different forms of endocarditis as follows: acute rheumatic, 61 per cent; recurrent rheumatic, 55 per cent; acute bacterial, 0 per cent; subacute bacterial, 8 per cent; old valvular defects, 10 per cent.

The calcified nodular type of old valvular defect may originate entirely independently of an inflammatory process, as Mönckeberg believes; but its frequent association with known inflammatory lesions has not been satisfactorily explained. It is noteworthy also that Aschoff bodies were found in the myocardium in three cases of this group.

Summary

In addition to the vegetations in acute endocarditis there is a diffuse inflammation always in the free edge and often involving the greater part of the leaflet. This circumstance explains the uniform thickening so commonly seen in old defective valves.

Rheumatic vegetations are composed chiefly of fibroblasts, and in the process of healing they readily become converted into fibrous tissue. There is no ulceration and no organization. Fifty-five of seventy-three old defective valves are considered the result of rheumatic endocarditis, and in twenty-seven of these incompletely healed rheumatic lesions were recognizable.

Bacterial endocarditis is a more intense inflammation than the rheumatic. Proliferation predominates but exudation is often prominent. Large thrombi are formed on the raw surfaces and there is often ulceration. Healing consists in the conversion of the leaflet into scar tissue. Such portions of the thrombi as do not become detached persist indefinitely without becoming organized, although they may become calcified. Complete healing rarely occurs. Three of seventy-three old defective valves were interpreted as the result of bacterial endocarditis.

Transitions between rheumatic and bacterial vegetations are frequently seen. Rheumatic vegetations were found in association with bacterial in three-fourths of the cases of subacute bacterial endocarditis.

Fifteen of seventy-three old defective valves belong to the aortic calcified nodular group. The etiology of this type is unknown. There is no satisfactory evidence that it is of inflammatory origin, and it seems unrelated to atheroma. Aortic stenosis in the absence of disease of any other valve is usually of this form.

Stenosis is more frequent than insufficiency in old defective valves.

The only old pulmonary valve defects seen were of the congenital type (three cases of pulmonary stenosis).

An acute rheumatic endocarditis may terminate in several different ways: (*a*) death during the acute stage from toxemia; (*b*) partial or complete healing followed after a variable interval by the reappearance of fresh rheumatic vegetations (recurrent rheumatic endocarditis); (*c*) partial or complete healing followed by the forma-

tion of bacterial vegetations on the valves — a more active inflammation (subacute bacterial endocarditis); (*d*) slow incomplete healing giving rise to deformed leaflets on which rheumatic inflammation is still recognizable; (*e*) complete healing resulting in thickened, stiffened valves with smooth surfaces.

As to pathogenesis, 76 hearts with old valvular defects are interpreted as follows: 55 from rheumatic endocarditis, 3 from bacterial endocarditis, 15 (all aortic stenosis of the calcified nodular type) of undetermined origin and 3 (pulmonary stenosis) congenital.

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DESCRIPTION OF PLATES

Plates 33–40

Plate 33

Fig. 1. 25–171. Acute rheumatic endocarditis. Ridge of small, firm vegetations near the edge on the auricular surfaces. Opacity of the leaflet above with prominent blood vessels and small hemorrhages.

Fig. 2. 22–28. Recurrent rheumatic endocarditis. Thickened leaflets with a ridge of very small vegetations near the edge on the auricular surfaces.

Fig. 3. 10–92. Early rheumatic lesion — area between vegetations. Endothelium intact, many large fibroblasts, a few lymphocytes and polymorphonuclears.

Fig. 4. 22–595. Early rheumatic vegetations. Closely packed fibroblasts. Abundant hyalin in one, a little in the other. A few polymorphonuclears and lymphocytes. Endothelium partly detached.

Plate 34

Fig. 5. 22–185. Rheumatic endocarditis. The multicentric character of the lesion is shown. Platelet thrombi have formed where the hyalin has broken through the endothelium. Under the vegetations there is a diffuse inflammatory reaction — many small fibroblasts and extensive perivascular lymphocytic infiltration.

Fig. 6. 22–185. Small vegetation composed largely of hyalin.

Fig. 7. 18–204. Recurrent rheumatic endocarditis. A typical well formed fresh vegetation. Surface hyaline layer. Edema. Many fibroblasts. Central capillaries. The valve underneath shows scarring from a previous attack.

Fig. 8. 10–92. Acute rheumatic endocarditis. Detail of surface vegetation. Surface hyaline layer. Zone of large fibroblasts. Serous exudate.

Plate 35

Fig. 9. 25–163a. Acute rheumatic endocarditis. Diffuse intense involvement of marginal part of leaflet. Hyaline layer on both surfaces. Numerous large fibroblasts.

Fig. 10. 25–163a. Higher magnification of an area of Fig. 9.

Fig. 11. 22–185. Acute rheumatic endocarditis. Cross-section of a leaflet showing hyalin in the central portion.

Fig. 12. 22–185. Acute rheumatic endocarditis. Section of valve above the level of the vegetations showing a diffuse proliferative inflammation. Large numbers of small fibroblasts. Prominent blood vessels. Some lymphocytes.

Plate 36

Fig. 13. 25–631. Recurrent rheumatic endocarditis. Active vegetations on a thickened scarred valve. There is a little cellular exudate in the scar tissue underlying the vegetation.

Fig. 14. 24–364. Acute rheumatic endocarditis. Vegetation in stage of healing. Scar tissue with retrogressive changes in fibroblasts. Homogeneous hyaline layer at surface.

FIG. 15. 17–227. Recurrent rheumatic endocarditis. Healed vegetation. The surface hyaline layer of the vegetation blends with the hyaline fibrous tissue.

FIG. 16. 17–28. Subacute bacterial endocarditis. Bacterial emboli in capillaries surrounded by a zone of polymorphonuclears (embolic abscess).

PLATE 37

FIG. 17. 18–102. Subacute bacterial endocarditis. Proliferative inflammation within the valve. Large fresh soft thrombus with extensive deposits of calcium in its deeper part.

FIG. 18. 21–513. Subacute bacterial endocarditis. Proliferative inflammation. Large closely packed fibroblasts (some multinucleated). Very few collagenous fibers.

FIG. 19. 13–180. Subacute bacterial endocarditis; stage of healing. Hyaline thrombus; no organization. Valve largely converted into scar tissue.

PLATE 38

FIG. 20. 25–328. Old valvular defect with incomplete healing. Ridge of vegetations near the margin of the thickened leaflet. See Fig. 21 for microscopic structure.

FIG. 21. 25–328. Section through the ridge of vegetations shown in Fig. 20. Dense scar tissue underneath an old hyaline thrombus. Very little evidence of organization. This may represent a healed bacterial lesion.

FIG. 22. 16–185. Old valvular defect with incomplete healing. Large ulcers covered by thrombus. Small denuded areas near the free margin. See Fig. 23.

FIG. 23. Section of the large ulcer shown in Fig. 22. Hyaline thrombus; very little organization. Many leucocytes within the valve. Healing bacterial lesion.

PLATE 39

FIG. 24. 15–324. Old valvular defect with incomplete healing. Denuded areas on the thickened leaflets.

FIG. 25. 11–121. Old valvular defect with incomplete healing. Rheumatic vegetation with a dense connective tissue core. The surface hyaline layer is easily seen.

FIG. 26. 25–328. Section of a healed vegetation composed of scar tissue. The superficial hyalin is visible on the left side. No organization.

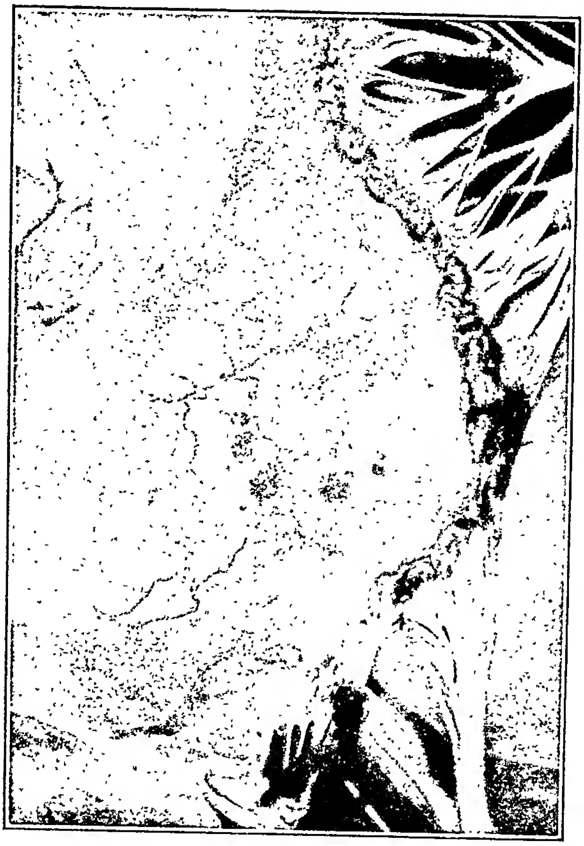
FIG. 27. 25–322. Old valvular defect with incomplete healing. Section through a raw area showing a thin layer of thrombus overlying dense fibrous tissue.

PLATE 40

FIG. 28. 20–250. Old valvular defect with incomplete healing. Old hyaline thrombus resting on hyaline scar tissue.

FIG. 29. 23–101. Old valvular defect with complete healing. Dense fibrous tissue in which a few capillaries are visible. A thin layer of hyalin at the surface suggesting the surface hyaline layer of a rheumatic vegetation.

FIG. 30. 25–82. Old valvular defect, calcified nodular type (Group 3). Aortic valve seen from above.



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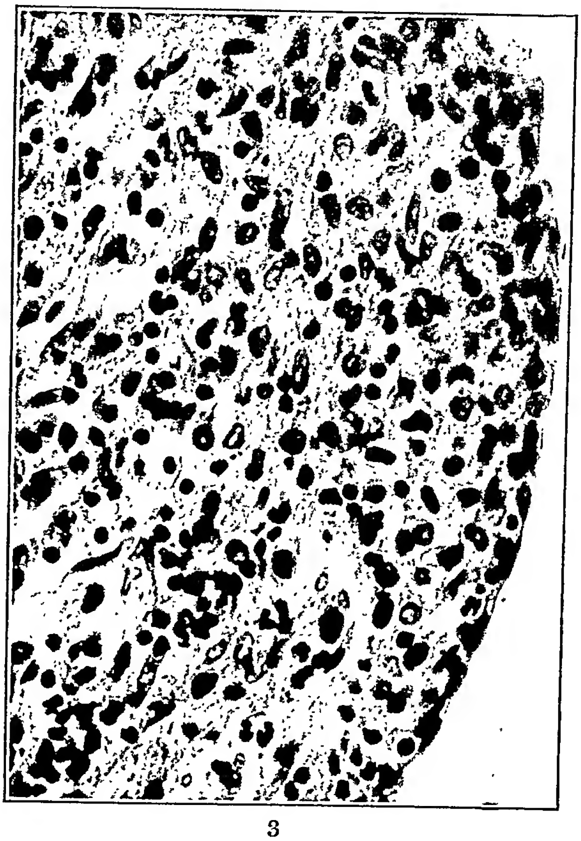

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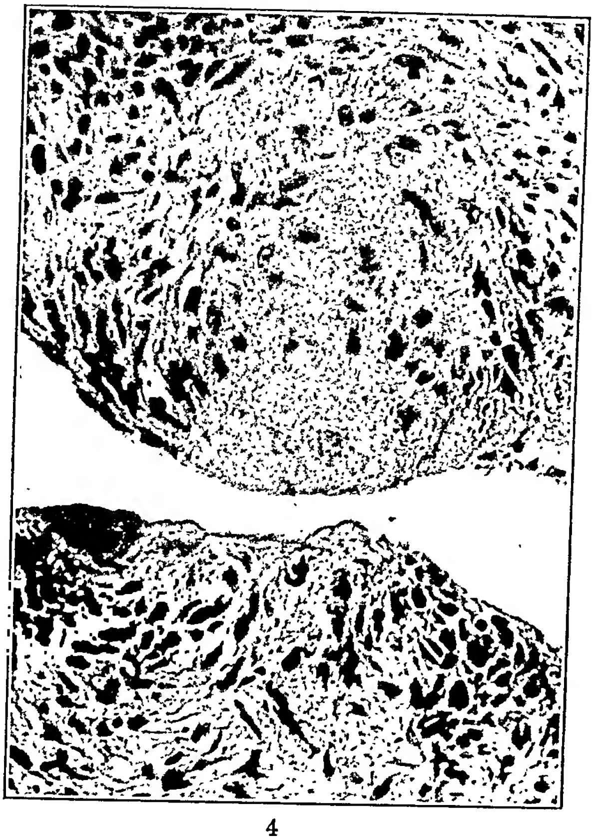

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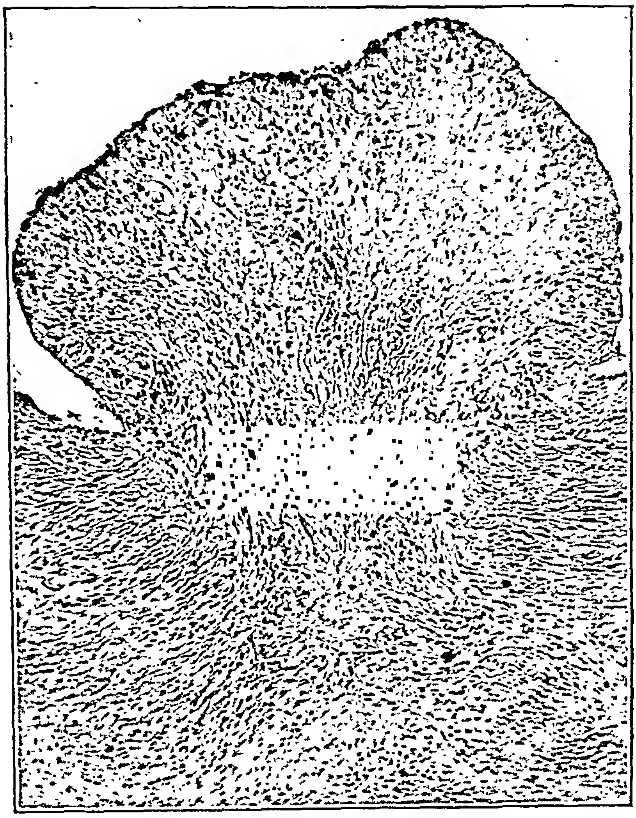

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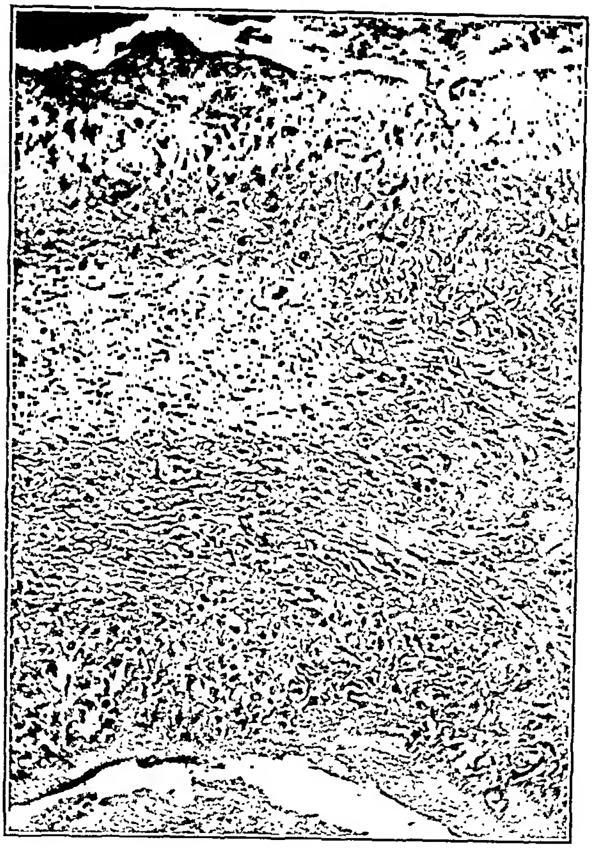
Valvular Diseases of the Heart



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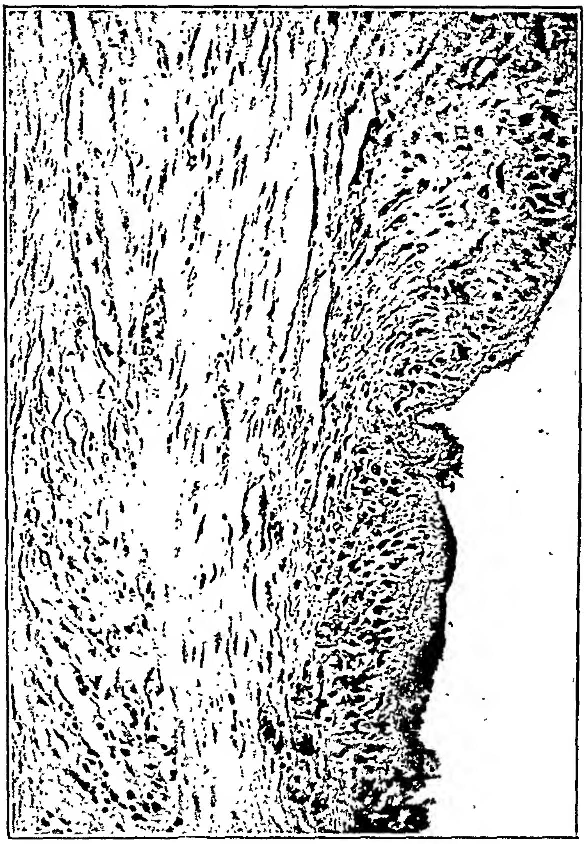
Valvular Diseases of the Heart



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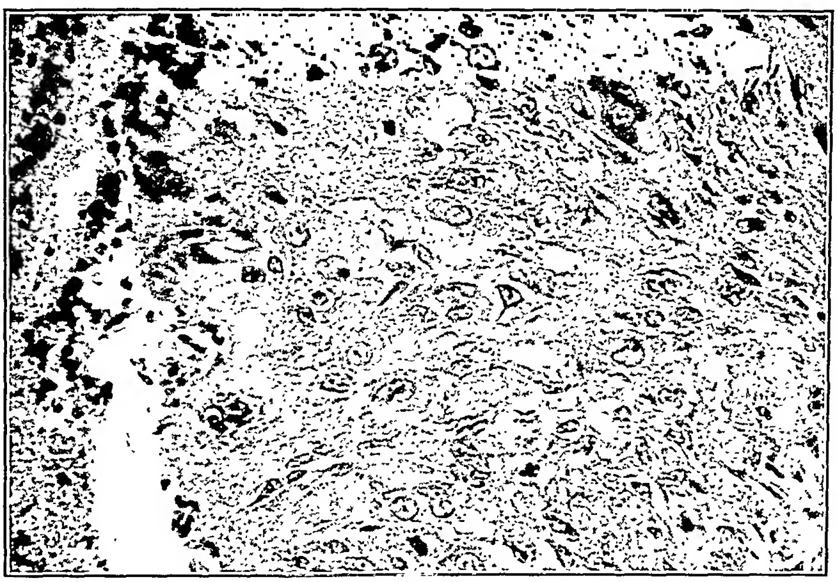

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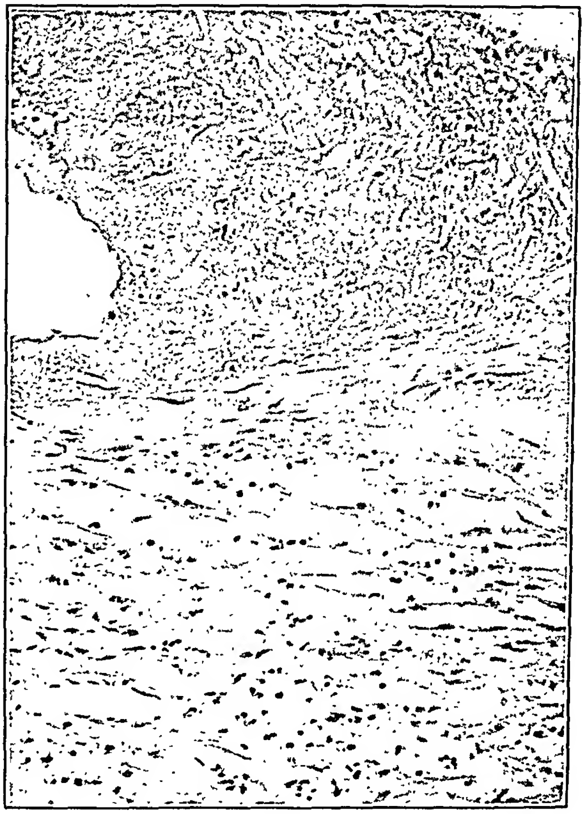

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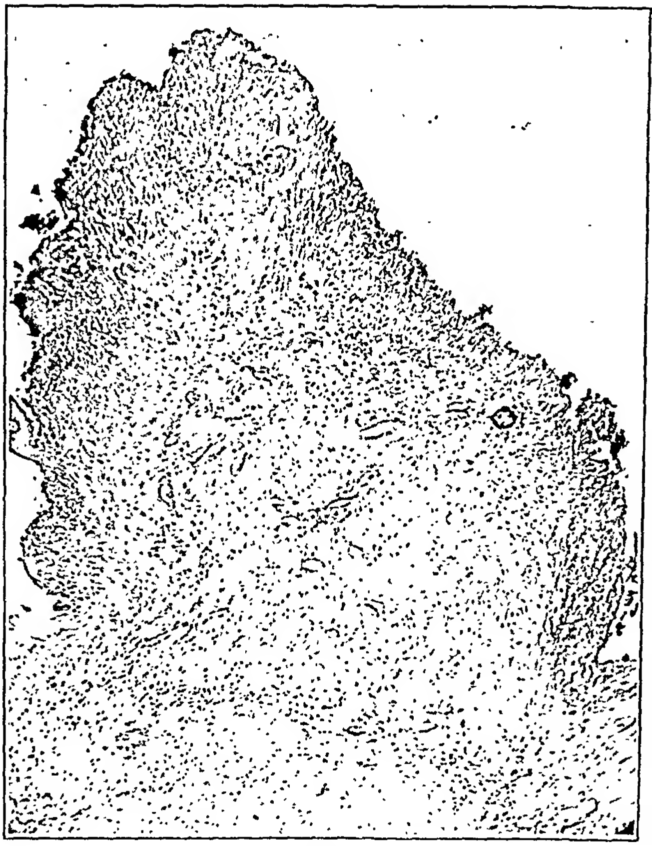

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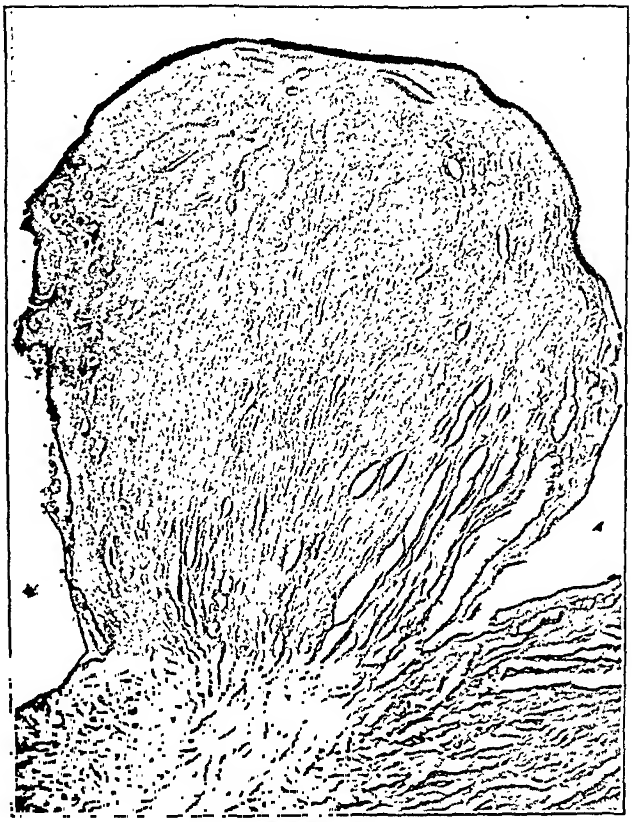

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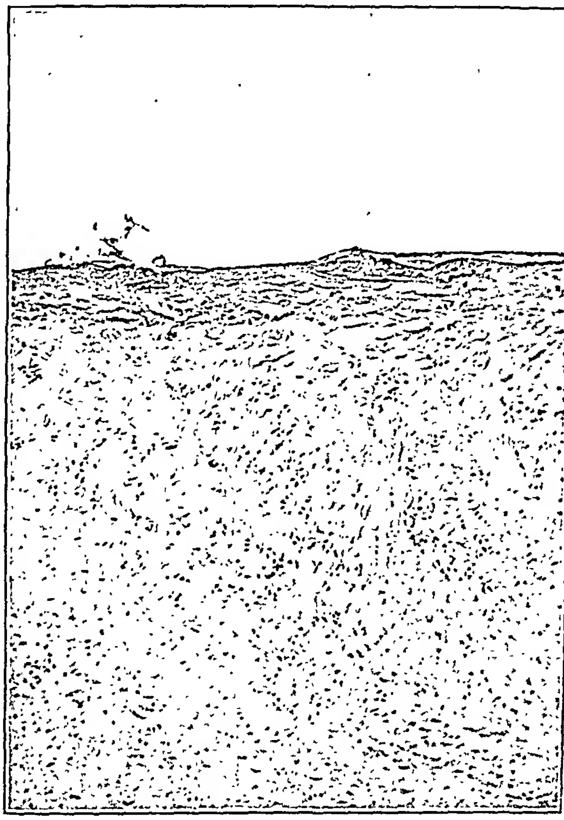

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Valvular Diseases of the Heart

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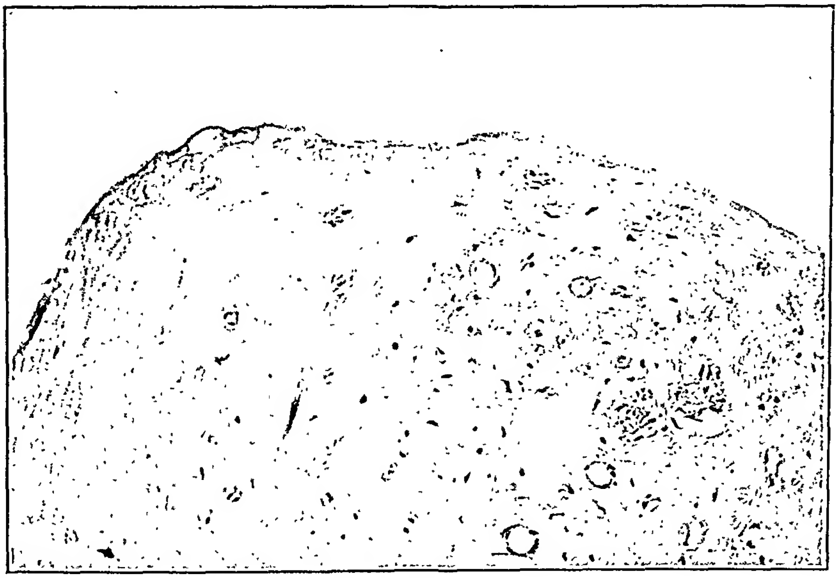

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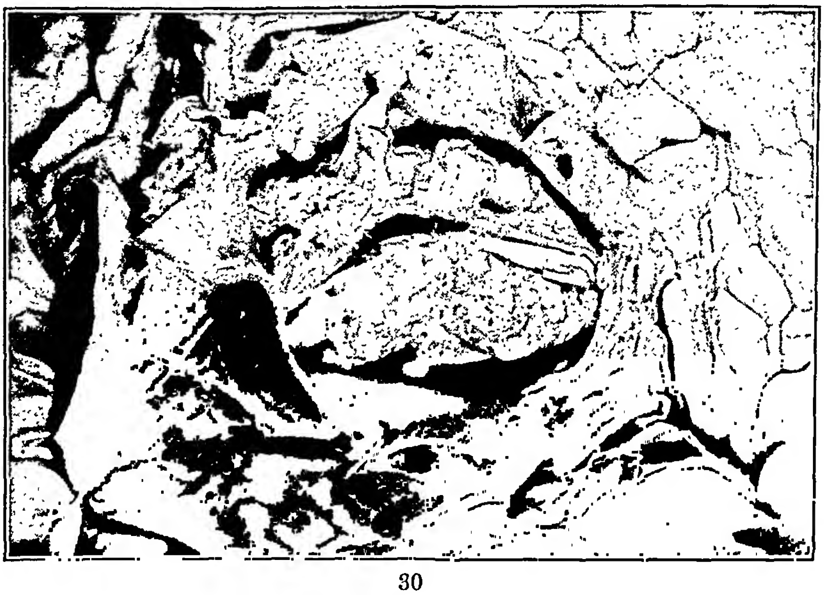

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SPECIFIC LESIONS OF PERIPHERAL BLOOD VESSELS IN RHEUMATISM *

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In the present uncertainty as to the causative agent of rheumatic fever, it is not possible to establish with finality the rheumatic nature of any given lesion. However, the frequent or constant association of a lesion with the clinical or pathologic features of rheumatic fever and its resemblance to other lesions generally recognized as associated with rheumatic infection, are presumptive evidence in favor of its rheumatic origin. Additional support is gained if the lesion in question has histologic features which are distinctive and which are not encountered in other known diseases. The specificity of the Aschoff nodule, indeed, of all rheumatic cardiac lesions, rests upon no firmer foundation than this.

That the virus of rheumatic fever may produce specific lesions of the aorta has been clearly shown.[1] It would not, therefore, be surprising to find that the smaller peripheral vessels should at times be the seat of rheumatic lesions. This in truth has proved to be the case.

A peculiar type of vascular inflammation has been found in a series of cases of rheumatic carditis, and in our experience in no other disease, so that we believe it to be specific and characteristic. We wish in this paper to describe the distinctive features of these lesions and to present detailed evidence in favor of their rheumatic origin. The material studied consists of a series of forty-seven consecutive cases of rheumatic heart disease, and of these the lesions were found in ten.

Distribution of the Lesions

The vascular changes to be described have thus far been found in the following situations: lungs, aortic valve, kidney, perirenal and periadrenal adipose tissue, appendix epiploica of the sigmoid colon, ovary, testis, pancreas and in a small polyp of the cecum. In most

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of these regions, only isolated vessels have been affected. As regards the lungs, however, two cases have been studied in which practically every small branch of the pulmonary arteries has been involved; in the *kidney* also the lesions have been quite widespread. The subcutaneous fat, joints and skeletal muscles have not been included in our routine material, so that we cannot speak of the possible occurrence of similar lesions in these tissues.

Histopathology of the Lesions

The alterations involve the entire thickness of the vessel wall and frequently, though not invariably, throughout its entire circumference.

The endothelium is swollen and basophilic, but appears intact. It may be exfoliated into the lumen (Fig. 1); this may in some instances take place after death while in other cases it is obviously lifted off by the accumulation of a coagulable exudate beneath it (Fig. 2). In no case has this alteration of the endothelium led to thrombus formation.

The wall of the vessel appears thick in comparison to the caliber of the lumen and this increase in thickness is particularly striking when only a portion of the circumference is affected. The thickening in the early phases of the lesions is due primarily to the infiltration of the vessel wall with fibrin (Fig. 3). This appears in the form of coarse interlacing strands staining pink with eosin, yellow with Van Gieson and blue with the Weigert fibrin stain. The fibrin threads in small vessels may extend into the contiguous cellular tissue, so that the original boundaries of the vessels are obscured (Fig. 4). In larger arteries the fibrinous exudate is often limited by the internal elastic lamella; in these cases, the threads are circumferentially disposed (Fig. 1).

Accompanying this deposition of fibrin, there occurs a necrosis of the cellular constituents of the vessel wall, as shown by the chromatin fragments scattered amongst the fibrin threads. In some vessels there is also extravasation of red blood cells, either immediately beneath the endothelium or in the meshes of the fibrin.

External to the necrotic wall of the vessel is a cellular tissue having a very distinctive and peculiar appearance (Fig. 5). It is composed of a loose fibrillar stroma, in part fibrinous, in which are many nuclei.

One may distinguish (1) lobed nuclei of polymorphonuclear neutrophiles, many of them pyknotic and fragmented, especially those nearest the vessel wall; (2) larger vesicular nuclei, staining less intensely than those of the polymorphonuclears and often distorted or compressed into bizarre elongate or club-shaped forms. They tend to be arranged radially. Still further out is a loose infiltration of lymphoid and plasma cells, occasional eosinophiles and young connective tissue cells. In this tissue are many dilated hyperemic capillaries, the largest often exceeding the diameter of the affected vessel. The zone of capillary distention frequently extends far beyond the area of cellular infiltration and is a constant and conspicuous feature of the early lesions (Fig. 10).

The behavior of the elastic fibers in the affected vessels can best be followed in serial sections. The earliest change noted in the internal elastic lamella is a swelling and partial alteration in the staining reaction, so that the fibers appear beaded and discontinuous (Fig. 1). As one follows the vessels into the region where there has occurred exudation of fibrin, the internal elastic lamella may become more difficult to distinguish (Fig. 11) and finally disappears altogether. Before this point is reached, one may observe that it has become greatly stretched and attenuated by the fibrin which has been deposited beneath the endothelium, and actual rupture often takes place, the ends becoming widely separated and everted. The gap between the ruptured ends is filled with a mass of fibrin.

The external elastic coat is even more difficult to trace, since in the small vessels affected it is often incompletely developed or even wanting. A few delicate fibrils persist and are pushed outward by the accumulated exudate (Fig. 11).

The recognition of a well formed wavy elastica interna, when the vessel is followed in series to a point where the injury is less severe, is evidence that the lesion may affect the small arterial branches.

In other instances, it is impossible even in serial sections to find any traces of an elastic coat, the thinness of the wall, as compared with the diameter of the lumen, indicating that the affected vessel is either a capillary or a precapillary venule.

Additional evidence that the lesion may affect capillaries was obtained in studying sections of an ovary. Here the vessel involved lay in the wall of a small cyst with the edema and cellular infiltration about it elevating the cyst wall into a rounded prominence which

projected into the cavity. When this vessel was followed in serial section, it could be traced directly into a sinusoidal capillary composed only of an endothelial lining with its basement membrane (Fig. 5). A similar observation was made in an affected capillary in the substance of the aortic valve.

It has been impossible to demonstrate bacteria in these lesions, either in the Gram-Weigert or methylene blue stained preparations.

In the larger arterioles of the lung the picture is slightly modified. The reactionary zone about the vessel is often inconspicuous or wanting, even when the infiltration of the media with polymorphonuclears is intense (Fig. 7).

Reparative Changes

The fibrinous exudate is gradually replaced by a permanent tissue. The fibrin filaments become swollen and fused into compact homogeneous masses which for a time retain the specific staining reaction. Isolated clumps of fibrin may be found even after the reparative process is well established.

Branching and polygonal cells with deeply staining vesicular nuclei, usually single but occasionally multiple (Fig. 11), appear amongst the fibrin threads. These we believe to be derived from the endothelium. Where there have been clefts or spaces left by the retraction of the fibrin, these cells tend to line them; and where extravasated red cells lie free in the meshes of the fibrin, the cells seem to encompass them and to establish new blood channels. There has never been observed any ingrowth of fibroblasts or newly formed capillaries from the adventitial tissue. This secondary vascularization of the intima is an amazing feature of the lesion. When fully developed, the original central lumen of the vessel which persists throughout, becomes surrounded by a spongy or vascular tissue composed of tensely congested, newly formed sinuses separated by a loose fibrous tissue. When followed in series, these newly formed channels are found to communicate in many situations with the original lumen on the one hand, and with collateral vessels on the other (Figs. 8 and 9).

The internal elastic lamella which has originally been displaced outwards by the exuded fibrin, may persist and may even become fortified by the development of a few fibrils which penetrate into the loose tissue between the newly formed sinuses.

While not frequently encountered, distended capillaries are occasionally seen lying between the internal and external elastic lamellae. These also may be followed through gaps in the internal elastic lamella to communicate with capillaries inside that layer.

The healed lesions thus resemble at first glance canalized thrombi. But it is clear when they are followed through their development that thrombosis does not occur at any stage. The resemblance is therefore only a superficial one. The fact that the circulation is at no time interrupted by thrombotic closure of the vessels explains the absence of infarcts in the lungs, even when the presence of an associated chronic passive congestion would favor their occurrence. Yet the interpolation of the cavernous tissue within the vascular tubes must bring about a slowing of the stream and probably results in stasis and congestion in the neighboring vascular channels.

The formation of new blood channels, as has been pointed out, seemingly depends upon an initial extravasation of red cells in the interstices between the fibrin strands. In some instances where this has not occurred, the development of new vessels fails to take place. In such a case, the exuded fibrin is gradually replaced by fibrillar connective tissue in which there eventually appear newly formed elastic fibrils. At this stage the picture simulates an obliterating endarteritis (Fig. 12).

The muscular coat in the larger arterioles is affected to a varying degree. In many instances, the muscle fibers virtually disappear, so that the internal elastic lamella when it has not undergone destruction comes to lie in close apposition to the elastica externa.

The fate of the peculiar inflammatory tissue which often forms a broad zone about the affected vessel is less easy to follow. Even in the more acute stages, many of the cellular components show degenerative changes, their nuclei being distorted and fragmented. Beyond that, it has not been possible to trace the process in detail. Presumably the wandering cells disappear. In our material there has not been observed, even in the healed lesion, great formation of scar tissue in the vicinity of the vessels.

Illustrative Cases

The following cases are chosen as exemplifying the lesions under discussion. A résumé of our material is given in abstract in Table I.

TABLE I

Autopsy no.	Age	Sex	Wassermann reaction	Rheumatic history			
				Tonsillitis	Arthritis	Cardiac	Chorea
9334	16	Male	o	+	–	+ Mitral stenosis and insufficiency	+ 10 years ago
9457	42	Female	o	–	+ "Growing pains" Polyarthritis, 3½ years ago	Heart trouble since childhood Mitral stenosis and insufficiency	–
9595	54	Female	o	–	–	Mitral stenosis and insufficiency Adherent pericardium	–
9626	33	Female	o	+	–	–	–
9627	9	Male	o	+	+ Frequent polyarthritis, first attack 4 years ago	Mitral stenosis Aortic insufficiency Pericarditis	–
9630	25	Male	o	+	+ Polyarthritis 2 years go	–	–
9636	47	Female	o	?	+ Polyarthritis First attack 10 years ago Admitted during second attack	+ Mitral insufficiency	–
9678	50	Male		?	+ Polyarthritis 10 and 3 years ago	+ Mitral stenosis	?
9680	9	Female		+	+ Polyarthritis 4 years ago	+ Mitral and aortic insufficiency	–
9691	68	Male	o	–	–	? Myocardial insufficiency	–

TABLE I

Lesions of rheumatic origin at necropsy	"Rheumatic" lesions of peripheral vessels	Other lesions	Blood cultures	
			Antemortem	Postmortem
Healed pericarditis Mitral stenosis	Pulmonary arterioles, acute and healing	Thrombus of right auricle Multiple infarcts of lungs Lobular pneumonia Fibrosis in lungs Chronic passive congestion of viscera	Negative (2)	Anhemolytic streptococcus (lung)
Mitral stenosis Rheumatic myocarditis (Aschoff bodies) Rheumatic aortitis	Bronchial and pulmonary arterioles, acute and healing Renal	Chronic passive congestion of viscera Myoma of uterus Hydrothorax Hydropericardium Ascites		
Adherent pericardium Rheumatic endocarditis, tricuspid, mitral and aortic valves Mitral stenosis Rheumatic myocarditis (Aschoff bodies)	Vessels about renal pelvis	Erysipelas Acute interstitial nephritis Acute splenic tumor Nodular cirrhosis of liver Lobular pneumonia Thrombi of left auricle	Negative	Negative
Rheumatic myocarditis (Aschoff bodies)	Ovarian Renal	Chronic nephritis Hypertrophy of heart		
Pericarditis Rheumatic endocarditis, mitral and aortic valves; left auricle Rheumatic myocarditis Rheumatic aortitis Aschoff bodies in diaphragm	Polyp of cecum	Chronic passive congestion of viscera Hydrothorax Ascites Polyp of cecum	Negative (3)	
Rheumatic endocarditis, mitral and tricuspid valves Myocardial fibrosis	Periadrenal fat	Lobar pneumonia Suppurative pleurisy	Pneumococcus I	
Rheumatic endocarditis, tricuspid, mitral and aortic valves Rheumatic pericarditis Rheumatic myocarditis (Aschoff bodies) Rheumatic aortitis	Perirenal fat Renal Aortic valve	Cholelithiasis Obsolete tuberculosis		
Rheumatic endocarditis, tricuspid, mitral and aortic valves Rheumatic myocarditis (Aschoff bodies) Rheumatic aortitis (Aschoff bodies)	Appendix epiploica of sigmoid	Thrombus of left auricular appendix Embolus of middle cerebral artery		
Rheumatic endocarditis (acute) Rheumatic myocarditis (Aschoff bodies) Rheumatic pericarditis	Perirenal fat	Subacute bacterial endocarditis Embolic nephritis Infarcts of spleen and kidneys		Anhemolytic streptococcus
Acute rheumatic myocarditis (Aschoff bodies)	Testis Pancreas	Thrombi of right auricle, and right and left ventricles Embolus of right internal carotid artery and right middle cerebral artery Infarct of brain		

CASE I. T. B., age 43, female (History No. 55714, Autopsy No. 9457, Dr. Butler). Admitted to Presbyterian Hospital in December 1922, and December 1923.

Past History. Acute rheumatic fever two and one-half years ago. Short of breath since childhood. Occasional precordial pain and palpitation for six years. No history of tonsillitis or chorea.

Present History. "Caught cold" four weeks before first admission. Fever, cough and malaise.

Physical Examination. Many coarse and fine râles in both lungs. Heart enlarged; soft systolic and a rough early diastolic murmur over entire precordium, loudest at apex. Pulmonic second sound accentuated. Liver edge 6 cm. below costal margin. Wassermann negative. Red blood cells, 3,500,000; white blood cells, 7,100; polymorphonuclear neutrophiles 86 per cent.

Discharged after three weeks with diagnosis of chronic bronchitis and chronic cardiac valvular disease (mitral stenosis and insufficiency).

Readmitted in December 1923, with history of bronchitis for ten days. Edema of legs.

Physical Examination. At that time, small areas of dullness and bronchial breath sounds near bases with coarse râles. Heart enlarged to right and left. Diastolic thrill at apex with systolic and diastolic shock. Rough rumbling diastolic murmur at apex and soft blowing systolic murmur, transmitted to axilla. Blood pressure 125 systolic and 68 diastolic. Lungs, dullness at apices with wheezing respiration. Small areas of dullness near bases with bronchial voice and diminished breath sounds. Coarse râles throughout lower two-thirds of both lungs. Liver edge 4 cm. below costal margin in midclavicular line. Shifting dullness in flanks with fluid wave. Extremities, marked edema of legs and ankles. Red blood cells, 3,600,000; hemoglobin, 80 per cent; white blood cells, 20,000; polymorphonuclear neutrophiles, 92 per cent.

Patient died on the second day after admission.

Clinical Diagnoses: Bronchopneumonia; chronic cardiac valvular disease (mitral stenosis and insufficiency); chronic bronchitis.

Necropsy. Anatomic Diagnoses: Chronic cardiac valvular disease, rheumatic (mitral stenosis); rheumatic myocarditis; cardiac hypertrophy and dilatation; hydropericardium; hydrothorax; ascites; edema of extremities; chronic passive congestion of viscera; acute arteritis, pulmonary arteries; fibromyoma of uterus.

Only the heart and lungs need to be described. The remaining viscera showed no lesions bearing upon the condition under discussion. Heart weighs 310 gm. Epicardium smooth. Right ventricle hypertrophied. Free edge of tricuspid valve thickened and opaque but without definite vegetations. Pulmonic valves normal. Left auricle dilated and hypertrophied. Auricular endocardium wrinkled above posterior leaflet. Mitral valve leaflets are shortened, thickened and calcified, projecting at right angles to blood stream. They are adherent to each other. Mitral orifice reduced to a buttonhole slit 14 mm. long. At two points the valve is ulcerated over calcium deposits. Chordae tendineae are thickened and shortened. Papillary muscles hypertrophied. Aortic cusps slightly thickened at their bases. Coronary arteries are normal. Myocardium shows no gross scarring.

Histologic Examination. Mitral valve: Greatly scarred with irregular hyaline masses of connective tissue and deposits of calcium. No bacteria are present in the Gram stain. *Myocardium:* Blocks from left ventricle are negative. A section taken through interventricular septum shows in the neighborhood of a

small coronary artery a few fusiform cells with vesicular nuclei, distinct nucleolus and purplish cytoplasm. There is fragmentation of collagen fibers in the vicinity of these cells. The appearances suggest a small Aschoff body. No scars are present. *Lungs:* Right lung weighs 500 gm. Lung is air-containing. On section it is reddish gray. Cut surface bathed in bloody fluid. Lower lobe contains numerous reddish areas which are relatively firm. Bronchial mucosa congested. Large branches of pulmonary artery project above cut surface. Alveolar septa thickened. No consolidation. Left lung weighs 400 gm. and presents same appearance.

Microscopic Examination. The most interesting changes are in the arterioles of microscopic dimensions. Practically no normal vessels are found. The lesions conform to those described and are illustrated in Figs. 2, 3, 7, 8, 9 and 12. All stages, from the subendothelial exudation of fibrin to final organization with development of new blood channels, may be seen.

There is no pneumonic exudate in the alveoli, but moderate edema and occasional fibrinous coagulum and desquamated cells are seen. There is congestion of the capillaries, but most intense about the affected arterioles. The pulmonary veins are normal.

The remaining viscera show only the changes of chronic passive congestion.

CASE II. E. H., age 47, female (History No. 62745, Autopsy No. 9636, Dr. Paige). Admitted to Presbyterian Hospital April 28, 1925, and died April 30, 1925.

Chief Complaints. Pain in left side for three weeks, multiple arthritis for two weeks, shortness of breath for ten years and hematuria for four days.

Past History. Acute rheumatic fever ten years ago. Increasing shortness of breath for three years. Influenza six months ago.

Present Illness. History vague and conflicting. The main points are (1) a known cardiac lesion; (2) polyarticular pain with fever for two weeks; (3) dyspnea; (4) hematuria for four days; (5) irregular menses; (6) headache. Has been irrational for three days.

Physical Examination. Obese woman, irrational. No petechiae. Suprasternal pulsation. Heart, considerably enlarged to left. Maximum impulse diffuse; heart sounds of fair quality, regular. Blowing systolic murmur heard over entire precordium, loudest over second right interspace. Blood pressure 170 systolic and 60 diastolic. Lungs dull at base; scattered moist râles. Abdomen, no masses felt. Right wrist somewhat reddened. No edema of extremities or clubbing of fingers. Red blood cells, 4,200,000; white blood cells, 24,800; polymorphonuclear neutrophiles, 90 per cent. Blood culture, sterile. Blood urea, 0.98 gm. per liter. Blood Wassermann, negative. *Urine:* specific gravity, 1.026; neutral; albumen, heavy trace; acetone present; many casts, epithelium and white blood cells; no red blood cells. Temperature 102.4 F; pulse 100; respiration 28.

Course. Eight hours after admission condition worse. Wildly irrational, then comatose. Râles in chest increased. Temperature gradually rose, death occurring in coma with temperature of 107.4 F.

Clinical Diagnoses: Acute rheumatic fever; chronic cardiac valvular disease (mitral insufficiency); cardiac hypertrophy; hypertension.

Necropsy. Anatomic Diagnoses: Rheumatic endocarditis (tricuspid, mitral and aortic valves); rheumatic pericarditis, fibrinous; acute rheumatic myocarditis; rheumatic aortitis; cardiac hypertrophy; chronic passive congestion of

liver, spleen and kidneys; edema of pia and lungs; focal necroses of liver; obsolete tuberculosis (right lung); cholelithiasis; melanosis of colon.

The lesions in this case bearing on the subject were in the heart and kidneys.

Heart weighs 500 gm. External surface and parietal pericardium covered with thick layer of fibrin, except over the posterior surface of the left ventricle. Many subepicardial hemorrhages. Myocardium pale and soft. No scars. Tricuspid valve irregularly thickened, with fusion of anterior and posterior leaflets. Pulmonic valve normal. Wrinkling of endocardium of left auricle above posterior leaflet of mitral valve. Mitral valve thickened, with tiny verrucae 1 to 2 mm. in size, of yellowish gray color along line of closure, and extending down to edge of valve and to insertion of chordae tendineae. Some of the chordae are slightly thickened. Papillary muscles hypertrophied. Aortic leaflets are thickened and have on them minute dull grayish yellow vegetations.

Histologic Examination. Recent fibrinous pericarditis without organization. Section of mitral valve shows characteristic rheumatic vegetations composed of hyaline material with ingrowing fibroblasts. Aortic valve also has characteristic vegetations; in its substance is a capillary, the wall of which is obscured by fibrinous infiltration. There is no great accumulation of cells about it.

Myocardium contains numerous Aschoff bodies and periarterial scars. (Culture of pericardial fluid sterile.)

Aorta in gross showed early atherosclerosis. Microscopically, there are scars about the nutrient vessels.

Kidneys, each weighs 110 gm. Identical in appearance. Normal on section save for congestion of vessels. Microscopically, no significant changes are found in the renal parenchyma. The arterioles and capillaries in the peripelvic fat and in the contiguous tissue of one kidney show an acute arteritis of the type described (see Figs. 4 and 10). There is necrosis and infiltration of all coats, but no suppurative changes. No thrombosis.

The viscera show no lesions of particular interest.

Discussion

One may advance the following arguments in favor of the rheumatic origin of these vascular lesions.

1. They have been invariably associated with undoubted cases of infection with the rheumatic virus and have not, in our experience, been found in any case which did not show rheumatic lesions.

2. The finer histologic features are in many respects analogous to those present in the rheumatic lesions of the left auricle, as described in the recent paper by VonGlahn.[2] Particularly in the reactive tissue about the vessels there is the same peculiar combination of elongate and distorted nuclei, eosinophiles and fragmented polymorphonuclears.

3. The lesions have a specific character and do not coincide with any hitherto well defined type of vascular disease. They perhaps most closely resemble those of periarteritis nodosa, but the following

differences may be noted: (*a*) In periarteritis nodosa, thrombosis is of frequent occurrence; this has not been seen in any instance in the rheumatic cases. (*b*) Periarteritis nodosa commonly affects arteries of medium caliber. In the cases above described the smaller arteries as well as those of medium caliber, arterioles and sinusoidal capillaries have been affected. (*c*) The inflammatory changes in periarteritis nodosa lead, as the name indicates, to nodule formation often of macroscopic dimension and frequently to the development of aneurysm and infarcts. The "rheumatic" lesions are not nodular and invariably are microscopic. Though the affected vessel may show slight dilatation, this is never sufficiently localized or sufficiently marked to justify description as an aneurysm. Nor, for the reason already discussed, do the "rheumatic" lesions lead to infarction or hemorrhages from rupture. The cellular components of the reactive tissue differ from those in periarteritis nodosa. In the rheumatic lesions, eosinophiles are not abundant; in periarteritis nodosa they are often the most conspicuous cellular element. Plasma cells also are not numerous in the rheumatic lesions; they are of frequent occurrence in the other.

The lesions bear even less resemblance to those occurring in the course of acute pyogenic infections. The absence of bacteria, of thrombus formation and of a typical suppurative reaction is sufficient to exclude vascular lesions of this category.

Review of Literature

It is most interesting that a localization of the rheumatic virus in peripheral vessels should have been surmised for many years. The term "rheumatic arteritis" dates back to the well known treatise of Bouillaud (1840),[3] and clinical cases purporting to illustrate this condition have been cited by Lemaire (1864),[4] Fernet (1865),[5] de Fajole (1866),[6] Lelong (1869),[7] Lecorché (1869),[8] Guéneau de Mussy (1874),[9] Legroux (1884),[10] Huchard (1892),[11] Hanot (1894),[12] Brault (1896),[13] Astier (1897),[14] Blot (1898),[15] Besson (1900),[16] Barié (1905, 1913)[17] and Queuille (1906).[18] In spite of their suggestive titles, little definite information can be extracted from these papers. For the most part, they are reports of clinical cases in which violent pulsation of the larger peripheral arteries attracted attention. The few necropsy reports and the still rarer histologic descriptions of the

larger vessels are too indefinite to justify analysis. A good summary of the French literature is to be found in the paper of Barié (loc. cit. 1913).

A few references, equally indefinite, are to be found in the German literature. Thus Wiesel and Löwy (1919)[19] in a paper upon the effects of acute and chronic circulatory insufficiency upon peripheral blood vessels, made the following statement (p. 1085): "Acute rheumatic fever likewise is accompanied not only by endocardial, but also by arterial lesions." * No specific changes are described. In a recent paper (1923), Wiesel[20] again alludes to the effect of rheumatism, as well as other infectious diseases, upon the peripheral arteries. Here he mentions changes in the media as a factor leading to juvenile arteriosclerosis. Fahr (1920)[21] is inclined to attribute to rheumatism, in addition to lues and lead, etiologic importance in the production of "malignant sclerosis" of the kidney. He cites five cases in which renal disease followed at varying intervals a rheumatic infection. No reference is made to distinctive vascular lesions. In a subsequent paper (1921),[22] Fahr again refers to rheumatic affections of the small renal arteries as a probable cause of malignant renal sclerosis. This paper contains a low-power photomicrograph of periarterial granulomatus formation with lymphoid cells, fibroblasts and occasional eosinophiles. The details are difficult to distinguish, but it is possible that the lesion pictured is identical with that described by us.

In the English and American literature no definite allusions to rheumatic arteritis have been found. However, it is not surprising to discover that Mallory,[23] who has interested himself particularly in infectious lesions of blood vessels, should have given in his textbook a most accurate reproduction of the type of lesion under discussion. The vessel involved was situated in the kidney, but there is nothing in the legend or text to indicate whether or not the case was one of rheumatic fever.

Ophüls,[24] in his discussion of the etiology of periarteritis nodosa, emphasizes the apparent close relationship between this disease and the "ill-defined group of subacute and chronic 'septic' conditions, with so-called rheumatic symptoms, and frequently associated with endocarditis." While it is possible that certain cases of rheumatic vascular disease may have been interpreted as periarteritis nodosa,

* "Auch die akute Gelenksrheumatismus ist . . . nicht nur von Erkrankungen des Endokards, sondern ebenso von solchen der Arterien begleitet."

there are, as has been pointed out, very clear-cut differential features. The final decision as to whether these two somewhat similar conditions are related, must be deferred until the cause of each is known.*

Conclusions

In a series of forty-seven consecutive cases of rheumatic cardiac disease, specific lesions of the small peripheral arterioles and capillaries, either systemic or pulmonary, have been found in ten.

These lesions are characterized by the exudation of fibrin into and about the vessel, by destructive changes in the cellular components of the vessel wall, by a distinctive cellular reaction in the adjacent tissue and by the absence of thrombosis.

These acute lesions are followed by organization with or without formation of new collateral channels within the thickened intima and occasionally within the muscular layer.

We wish to express our thanks to Doctor Walter W. Palmer for permission to use the clinical records, and to Mr. Alfred Feinberg and Miss Madeline Chickering for the drawings.

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* There has come to our attention since this paper was submitted for publication, an article by Baehr and Sacks (*Proc. New York Path. Soc.*, 1923, xxiii, 64). They described vascular lesions of the renal arterioles in cases of glomerulonephritis associated with verrucous endocarditis, three of which were regarded as typically rheumatic, and two as belonging to the "atypical form" described by Libman and Sacks.

In the mildest form of involvement, the changes . . . "consisted of endothelial swelling and proliferation. In the more severely damaged arterioles, there was irregular hyaline degeneration and necrosis of the normal elements of the vessel wall, with karyorrhexis of many of the nuclei, infiltration at times with polymorphonuclear and round cells, and the deposition of fibrin and blood platelet thrombi in the lumen. These changes were often limited to the vasa afferentia close to the pedicle of the glomerulus; in other instances they extended as far as the terminal segments of the interlobular arteries. Some of the arterioles showed thrombus deposition with comparatively little inflammatory change in the vessel wall."

The authors are undecided whether these cases constitute a specific infection with an endotheliotropic virus, unusual forms of rheumatic fever or are related to the atypical group of endocarditis of Libman and Sacks.

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DESCRIPTION OF PLATES

Plates 41–46

Fig. 1. Autopsy No. 9680. Arteriole in fat near pelvis of kidney. Exfoliation of endothelium and necrosis of vessel wall with fibrinous exudation. Marked inflammatory reaction in adjacent tissue. Congestion of surrounding capillaries. (Hematoxylin-eosin stain.)

Fig. 2. Autopsy No. 9457. Pulmonary arteriole. Fibrinous exudate beneath endothelium. Stretching and beginning fragmentation of internal elastic lamella. Thinning of muscular coat. Slight inflammatory reaction in surrounding tissue. No thrombus formation. (Weigert's elastic tissue-hematoxylin-eosin stain.)

FIG. 3. Autopsy No. 9457. Pulmonary arteriole. Dense accumulation of fibrin beneath endothelium with small masses in muscular coat. (Gram-Weigert-safranin stain.)

FIG. 4. Autopsy No. 9636. Arteriole in column of Bertini of kidney. Fibrin beneath endothelium, in vessel wall and ramifying into surrounding tissue. (Gram-Weigert-safranin stain.)

FIG. 5. Autopsy No. 9626. Sinusoidal capillary in wall of ovarian cyst. Necrosis of vessel wall with fibrinous exudate. Peculiar type of avascular inflammatory tissue surrounding the vessel. (Hematoxylin-eosin stain.)

FIG. 6. Inflammatory tissue in wall of left auricle in rheumatic endocarditis. (Reproduced from "Endocarditis of rheumatic origin," VonGlahn, Fig. 10, Am. J. Path., 1926, ii, 1.) Compare character of cell reaction with that shown in Fig. 5.

FIG. 7. Autopsy No. 9457. Pulmonary arteriole. Acute lesion in cross-section and healing state in portion of vessel cut longitudinally. Zone of capillary congestion about affected vessel. (Wiegert's elastic tissue-hematoxylin-eosin stain.)

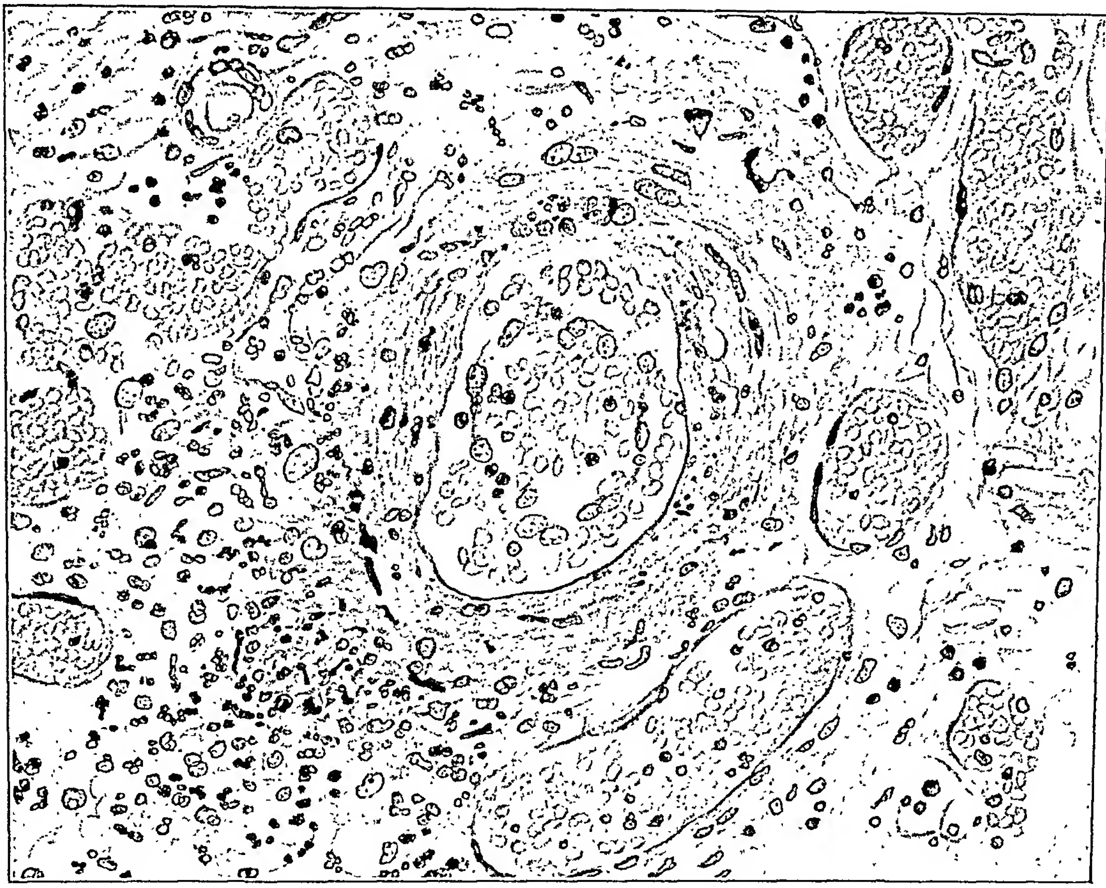
FIG. 8. Autopsy No. 9457. Pulmonary arteriole. Late stage, longitudinal section of vessel. Lumen occupied by distended capillaries surrounded by loose connective tissue. The elastic lamella has remained intact. (Weigert's elastic tissue-hematoxylin-eosin stain.)

FIG. 9. Autopsy No. 9457. Pulmonary arteriole. Cross-section. Changes same as in Fig. 8. (Weigert's elastic tissue-hematoxylin-eosin stain.)

FIG. 10. Autopsy No. 9636. Arteriole in column of Bertini of kidney. Necrosis of vessel wall with fibrinous exudate. Necrosis of elastic lamella. Note zone of capillary congestion about affected vessel. (Weigert's elastic tissue-hematoxylin-eosin stain.) Same vessel as shown in Fig. 4.

FIG. 11. Autopsy No. 9691. Arteriole in testis with internal elastic lamella almost completely destroyed. A portion of it persists immediately near upper margin of original lumen. Another fragment is seen below separated from lumen by large mass of fibrin; a few fragments remain also of the external elastic lamella. The fibrinous mass contains a number of large basophilic branching cells with one or several nuclei. There is also a newly formed blood channel. Lesion shows repair. (Weigert's elastic tissue-hematoxylin-eosin stain.)

FIG. 12. Autopsy No. 9457. Pulmonary arteriole. Healed stage without vascularization of fibrinous exudate. In this stage the vessel presents the picture of obliterating endarteritis. Two fragments of the internal elastic lamella are shown marking the limits of the original intima. (Hematoxylin-eosin stain.)



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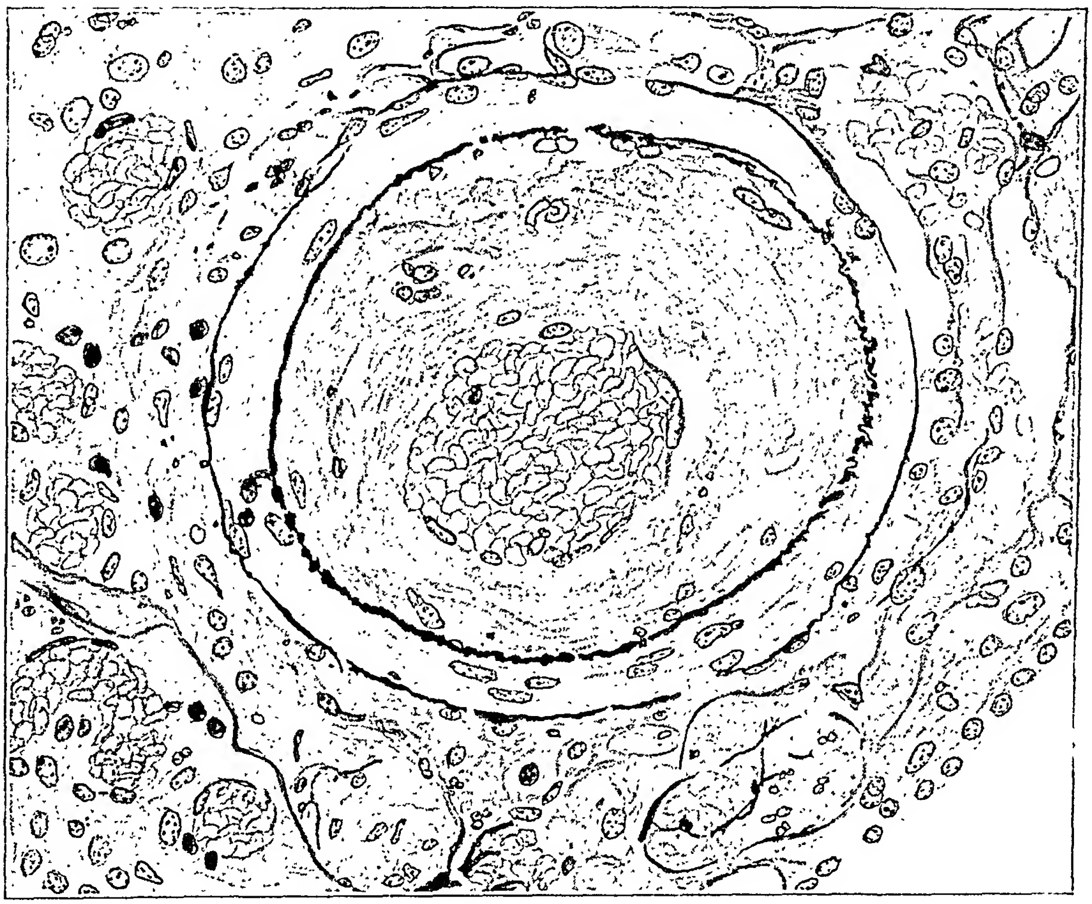

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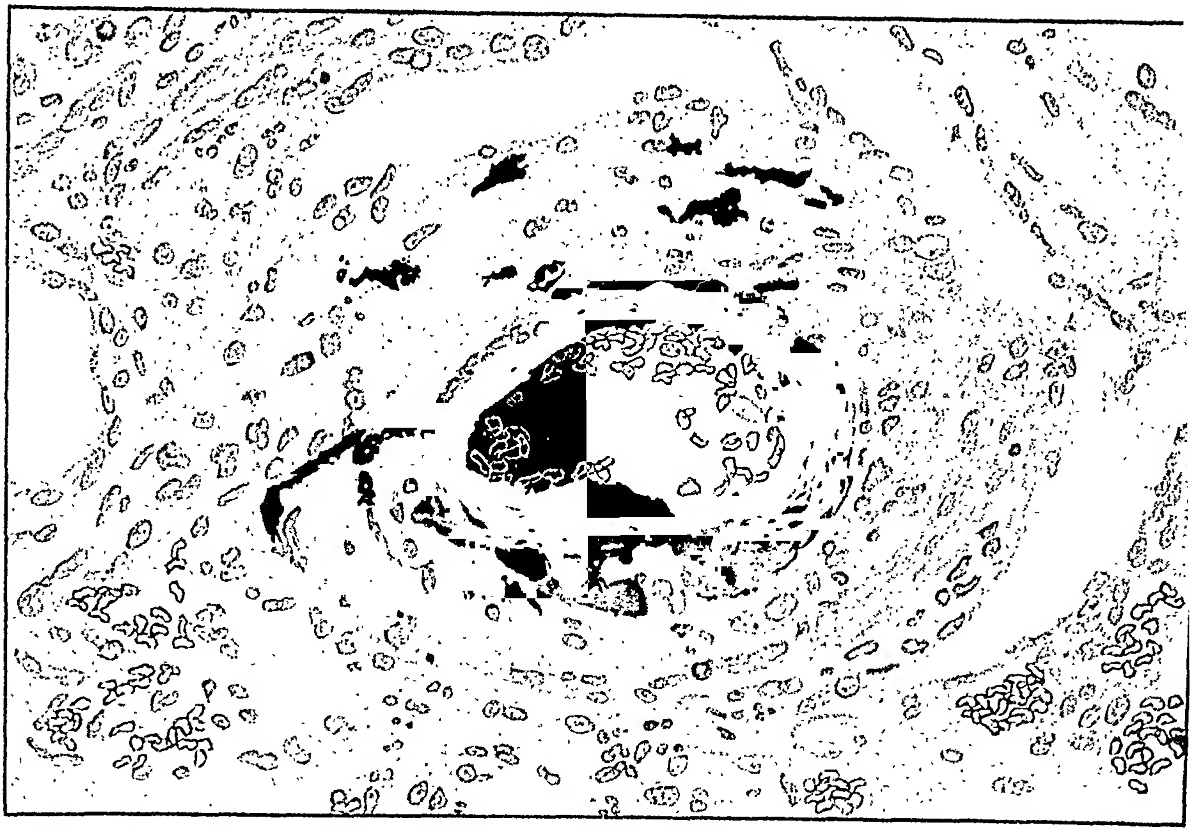

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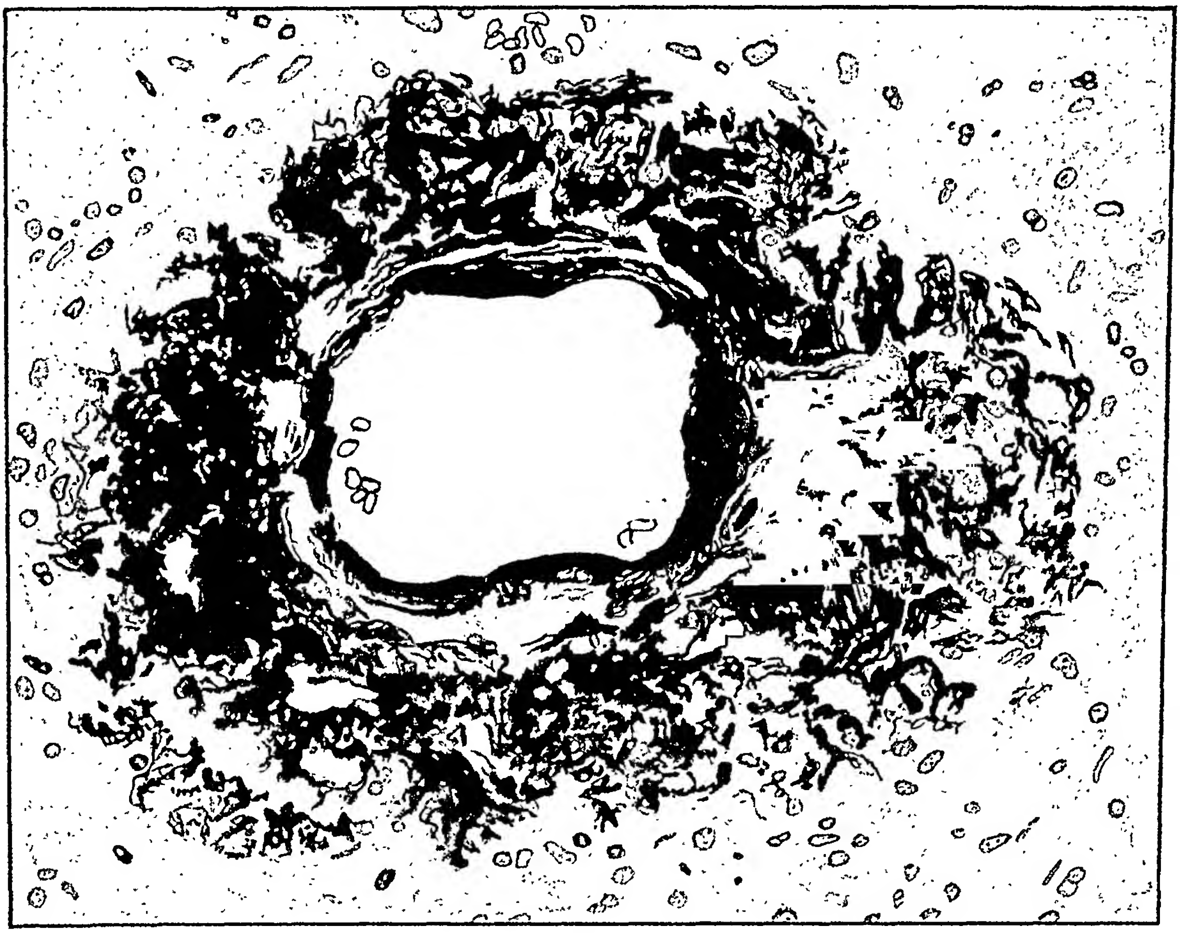

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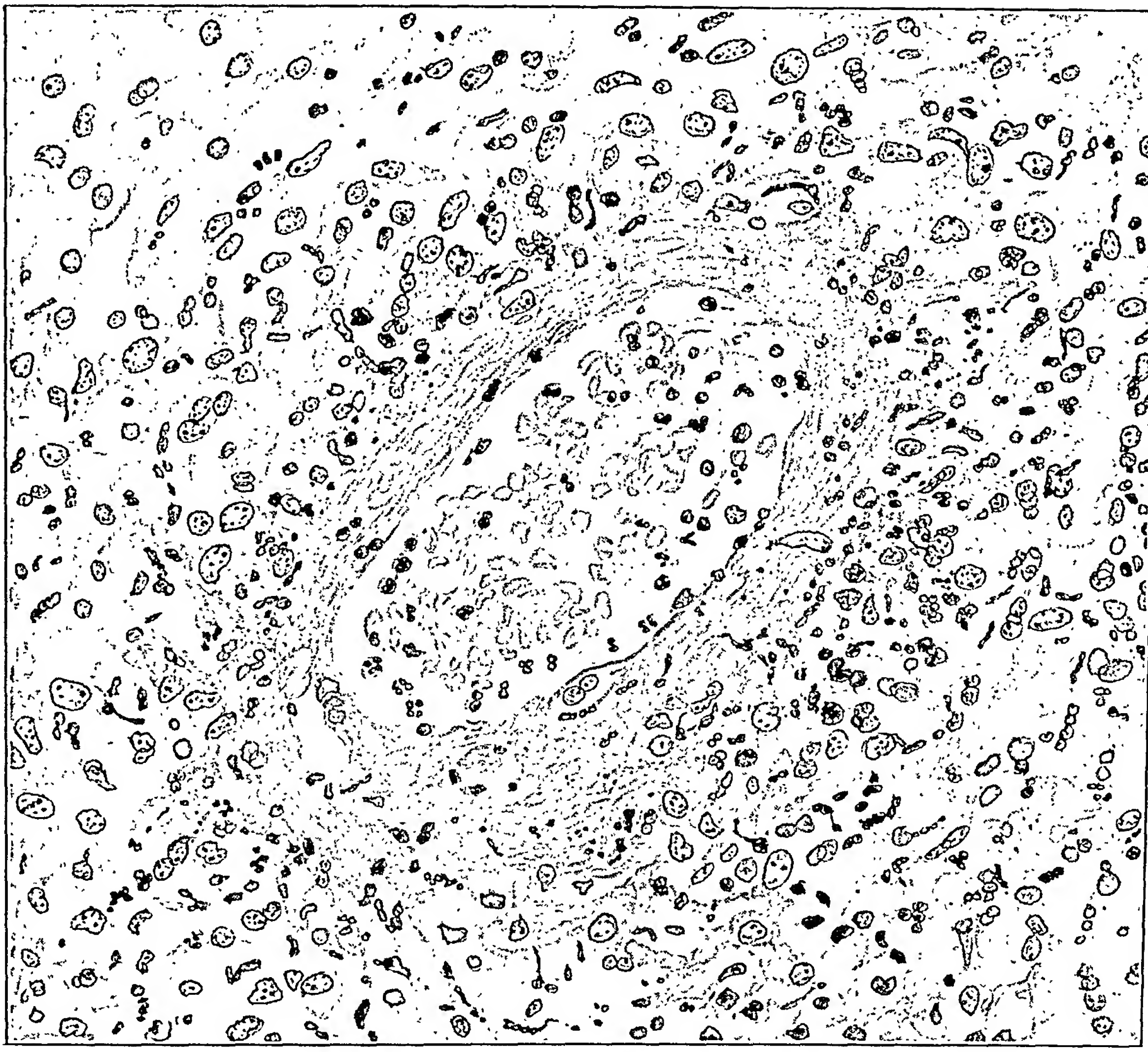

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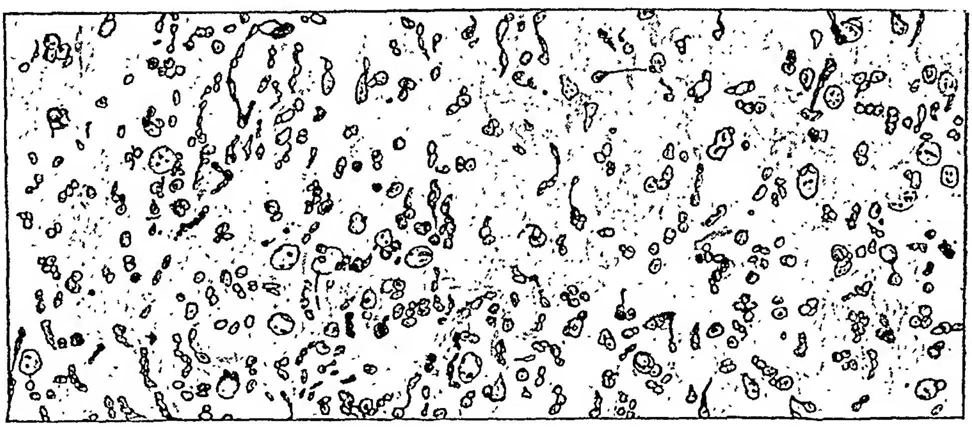

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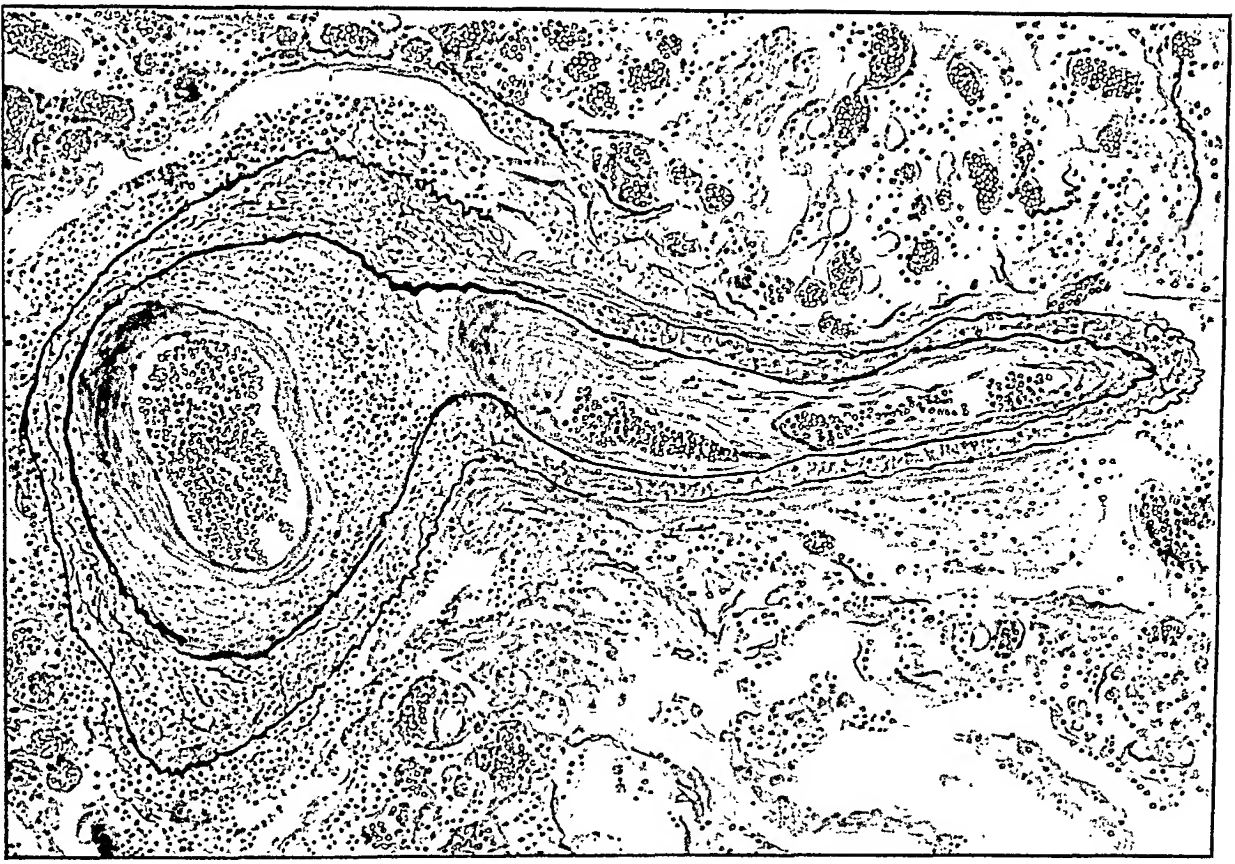

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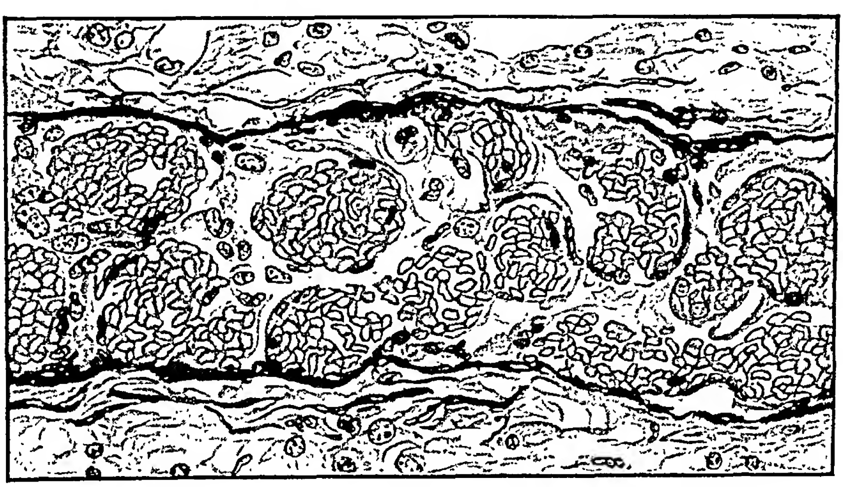

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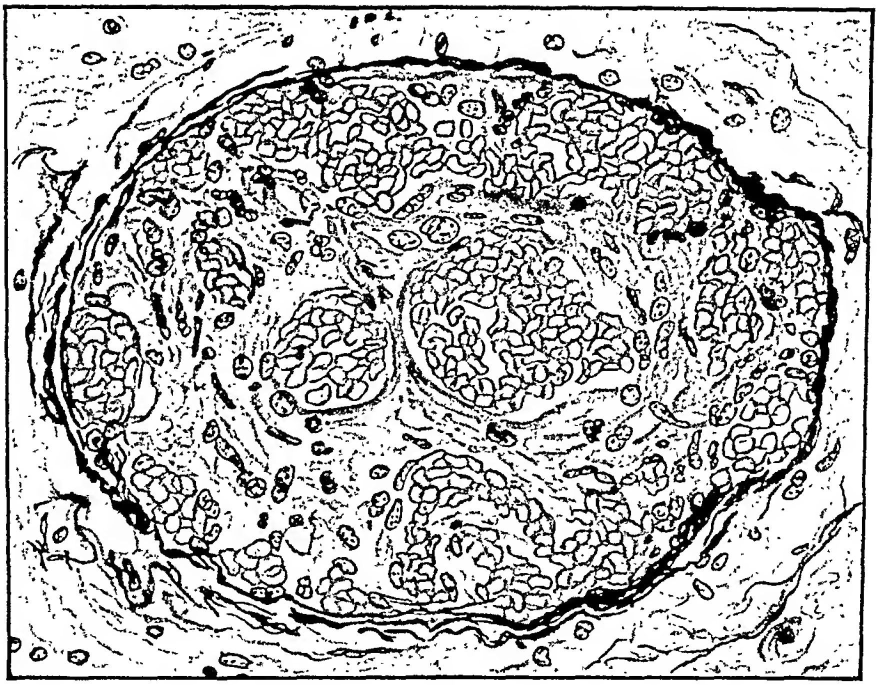

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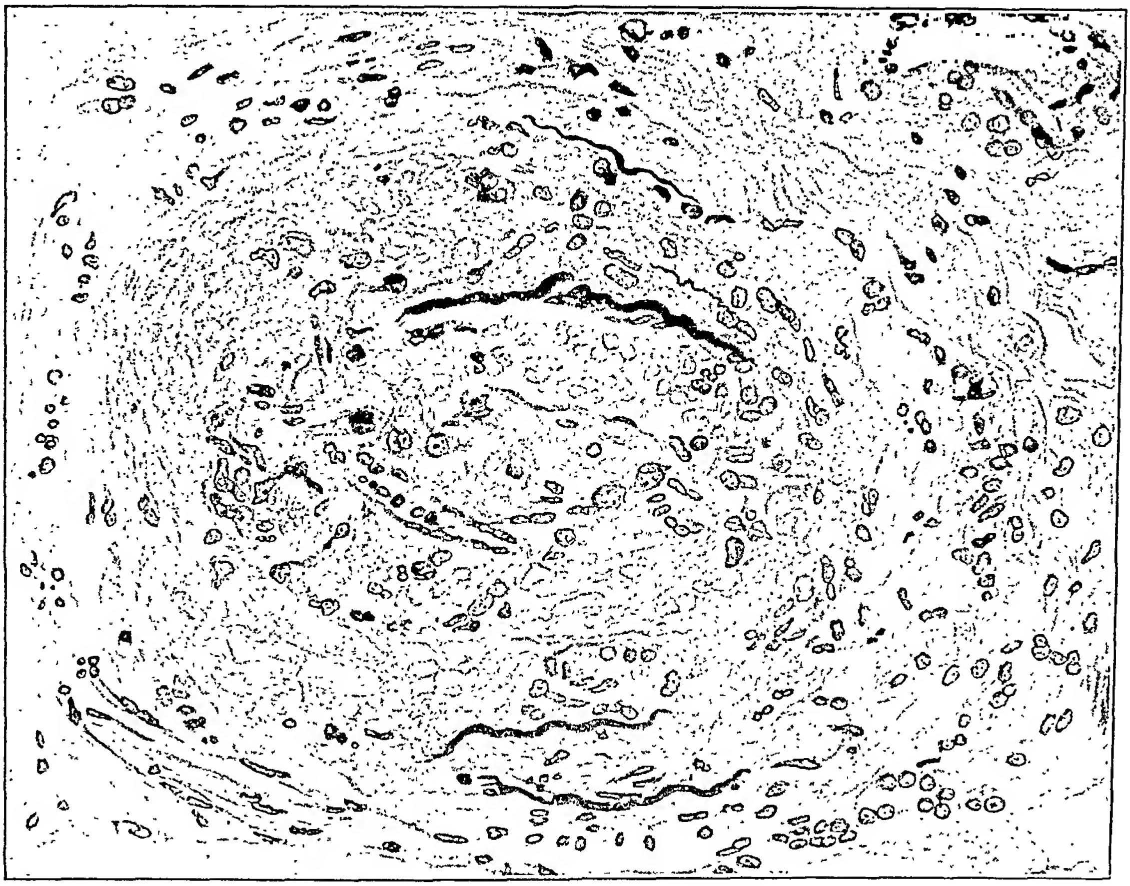

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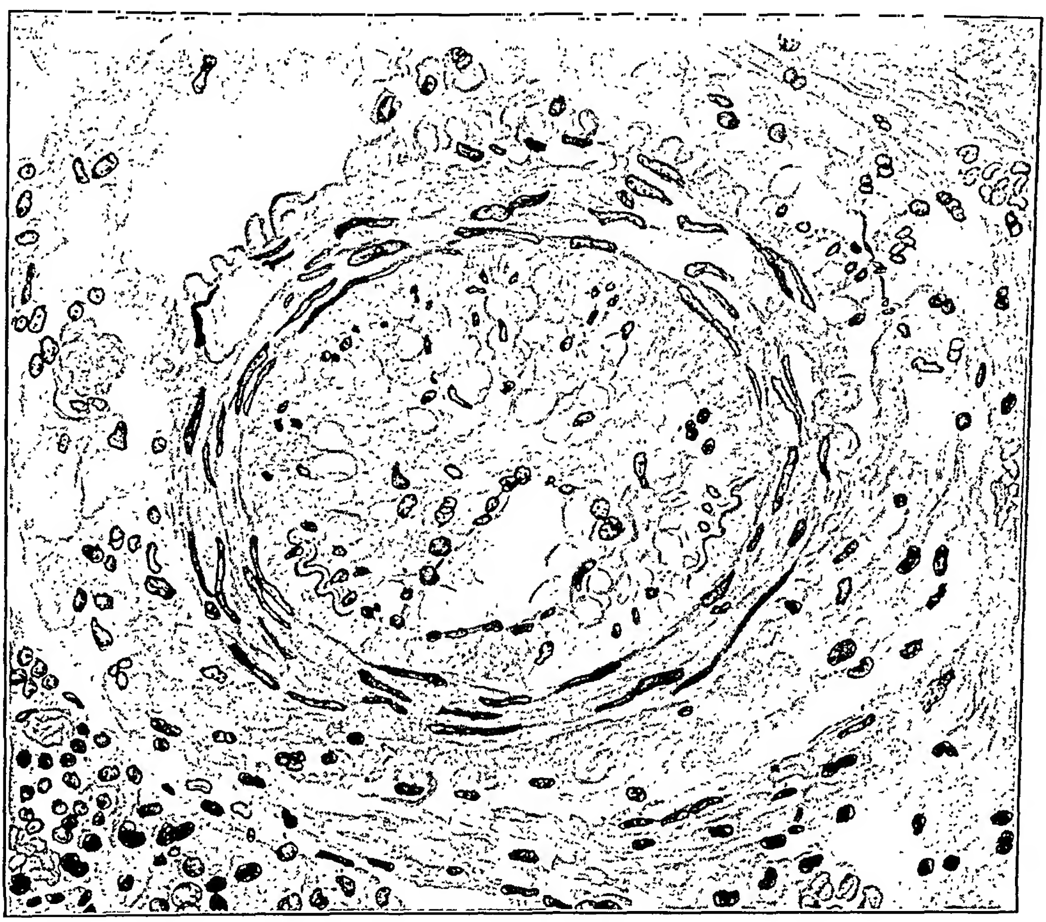

12

A CASE OF ADIPOSIS DOLOROSA WITH NECROPSY *

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Introduction

Adiposis dolorosa, first described by Dercum,[1] is characterized by the following syndrome: (1) great and localized adiposity with tenderness to pressure over the fat pads and along the course of nerves, occasionally with pain and aching in the extremities; (2) marked asthenia, or fatigability; (3) psychoses and epileptiform seizures; and (4) bullae or ulcers on the extremities. Subnormal temperature, decreased metabolic rate and a tendency toward hemorrhage may also be present. The disease is characterized pathologically by changes in the endocrine organs, such as sclerosis with atrophy in some instances and hypertrophy in others. There is much to indicate that the primary lesion is situated in the hypophysis cerebri, the other changes occurring consequently, but the character of this lesion is very variable, as will be seen presently.

The case we are reporting fulfills the above criteria, but as there is no manifest history of tenderness over the fat masses we may be criticized for including it in the category of painful adiposity. It is, however, not improbable that the patient might have admitted tenderness over the masses had we been able to question her on that subject and it is our impression that this symptom, during life, is of far less importance than the conformity of the pathologic findings in the case to those previously described.

Winkelman and Eckel[2] recently reported an instance of this disease, with necropsy, and here the reader may find a complete synopsis of the fifteen cases reported prior to theirs, as well as a list of references. Although some two hundred clinical reports have been made, only sixteen necropsies appear in the literature. Winkelman and Eckel review the subject as follows.

> On reviewing the necropsy findings of the fifteen cases in the literature, one is impressed by the pluriglandular involvement in most of the cases. In only

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two cases were there no definite changes in the ductless glands (Rome and Falta). Of the eleven patients in whom the pituitary body was examined, eight showed definite alterations; the thyroid was abnormal in twelve cases; the sex glands were pathologic in nine; the suprarenal in three; and the pancreas in two. Our own case showed pituitary, suprarenal and ovarian pathologic changes. It is of interest from a clinical standpoint, to note that the condition is five times more prevalent in the female and that most cases develop after the age of 35.

The changes in the pituitary gland, as summarized in this review, vary from slight lymphocytic infiltration to adenomatous hyperplasia, or to malignant transformations constituting actual adenocarcinoma, or resembling it. One glioma is recorded. There is, then, no typical pituitary lesion. The usual change in the thyroid is sclerosis, with or without calcification and colloid retention. The suprarenals showed unilateral hyperplasia or adenoma in three instances. The gonads were listed as atrophic, sclerotic or "defective" in the nine reports mentioning them. Sclerosis and fatty invasion of the pancreas were noted in a few instances. Having stated the general characteristics of this disorder, let us proceed to the description of our case.

Clinical History

Present Illness. Ida F., 60 years of age, colored, single and a cook by occupation, was admitted to the medical service of the Cincinnati General Hospital on the evening of December 30, 1925. As she was irrational, non-coöperative and excited and as she died on the following afternoon, it was impossible to procure a connected history or to make a satisfactory examination of the patient. She had been an inmate of the Hamilton County Home for about two and a half years and had been granted leave to spend the Christmas holidays with friends in Cincinnati. While in apparently good health, she suddenly had a "stroke" which, so far as can be ascertained, consisted of a state of unconsciousness of unknown duration. Since then she seemed "out of her head" and was brought to the hospital the day following her seizure.

Past History. Only one fact seems fairly certain: she had been fat all her life, a childhood friend stating that she weighed over 200 lbs. when only 18 years old. She suffered from no severe illnesses and was always up and about, although recently inconvenienced by leg ulcers, shortness of breath and asthenia. While walking outdoors, she would frequently be forced to sit down on a door step and catch her breath and rest before being able to proceed. While at the Home she could always care for herself and at no time had anyone heard her complain that her fat was painful.

Family History. Her parentage was obscure; she seems to have had no brothers or sisters and was reared by an aunt who was likewise "very heavy."

Physical Examination. This was very unsatisfactory on account of the patient's mental condition and her extreme obesity. The positive findings include large, equal and fixed pupils and a bitten, bleeding tongue-tip held be-

tween a toothless upper gum and a few worn, carious lower teeth. Percussing the chest, and locating the point of maximum impulse and relative cardiac dullness were equally unsatisfactory and uninstructive, on account of the heavy layers of fat over the chest. Besides labored breathing, auscultation revealed muffled, rather harsh and prolonged expiratory sounds. The faint, irregular heart sounds presented many extra systoles; the blood pressure did not register and the radial pulse was impalpable. Varicose ulcers were on both legs.

Laboratory Data. Temperature, 97 F; pulse, 96 (?); and respiration 28. Blood: erythrocytes normal and leucocytes 15,500 per c. mm. A differential count showed: neutrophiles 68 per cent; eosinophiles 0 per cent; basophiles 1 per cent; lymphocytes 30 per cent; large mononuclears and transitionals 1 per cent. Blood chemistry: sugar, 165 mgm.; urea nitrogen, 34 mgm.; and alkaline reserve, 42. Wassermann, negative.

Urine: A catheterized specimen was amber, clear and acid to methyl red; albumin, +++; sugar, positive; acetone, negative; blood (by Meyer's test), ++++. Several hyaline and granular casts, a few leucocytes and erythrocytes were found in the sediment.

Course in Hospital. Until she died, less than a day after admission, the patient had six convulsions, lasting from two to five minutes and preceded by a peculiar outcry. The whole body was equally involved, first in a short tonic, then in a clonic spasm lasting two to three minutes and followed by a gradual relaxation. During the latter part of each seizure and just afterward, she frothed at the mouth. During the day Cheyne-Stokes respiration set in and slight right-sided facial weakness was noted, although the extremities were not involved.

Clinical Diagnoses: Uremia, cerebral hemorrhage (?), auricular fibrillation and adiposis dolorosa.

Necropsy Protocol

Anatomic Diagnoses: Adiposis dolorosa. Bilateral suprarenal adenomas and softening, thyroid hyperplasia and sclerosis, persistent thymic tissue, atrophy and fatty invasion of pancreas, atrophy of the ovaries and adenomatous hyperplasia and sclerosis of the hypophysis with widened sella turcica. Exostoses of the inner table of the skull, tumors of the dura, cerebral atrophy and increased cerebrospinal fluid.

Cardiac hypertophy and dilatation, fatty infiltration of the myocardium with myocardial degeneration, fatty invasion from the epicardium. Atheroma of aorta and arteriosclerosis. Hypostatic pulmonary congestion. Atrophy and fibrosis of the spleen. Fatty invasion and passive congestion of the liver and cholelithiasis. Chronic vascular and glomerulonephritis. Fibrosis of the uterus and uterine polyp. Umbilical hernia. Ulcers of both legs above the ankle. Dental caries and pyorrhea alveolaris.

Cause of Death: Uremia and cardiac failure from decompensation.

DESCRIPTION OF NECROPSY

The body is that of an extraordinarily adipose negress (Fig. 1), measuring 161 cm. in length and weighing approximately 350 lbs. (77 kgm.). Rigor mortis is marked in the extremities and the lower jaw; no postmortem lividity can be determined. The fat is apparently subcutaneous and is deposited in huge accumulations over the abdomen, shoulders, chest, upper arm and thighs; while the head, forearms and hands and the lower legs and feet are of normal size and free from it. A thin, brownish fluid escapes from the mouth; the lips are cyanotic. The pupils are equal and about 4 mm. in diameter, with marked arcus senilis of the irides. The right radius exhibits a knob-like protuberance over the inner distal extremity, suggesting an old, healed fracture. There is a delicate grayish blue striation of the skin over the flanks (striae atrophicae) and glazed and thickened skin (intertrigo) over the apposed surface of the apron-like abdominal fat pad and that of the thighs, more than half-way to the knees. The fatty deposits are quite symmetrically distributed in every instance. Ulcers present over the inner surface of both shins, just above the ankle; that on the right measures 9.5 × 2 cm. and that on the left 2.5 × 3 cm. The surrounding skin is atrophic, scarred and shows varicosities; the ulcers are covered by apparently healthy granulations.

Body Measurements

	In circumference		In circumference
Head	57.0 cm.	Waist	142 cm.
Upper arm	58.0 cm. each	Hips	150 cm.
Forearm	right, 36.5 cm.	Thigh	right, 85 cm.
	left, 38.0 cm.		left, 90 cm.
Wrist	right, 21.0 cm.	Knee	51.5 cm. each
	left, 20.0 cm.	Calf	right, 45 cm.
Chest	125 cm.		left, 46 cm.
Bust	143 cm.	Ankle	25 cm. each

	Thickness	Length	Width
Hands	5 cm.	17.5 cm.	Average, 11 cm.
Feet	7 cm.	26.0 cm.	10 cm.

Iliac crest to knee	54.5 cm. on each side
Knee to internal malleolus	39.0 cm. on each side
Length of arm	40 cm. each
Length of forearm	30 cm. each

The primary incision reveals a thick layer of subcutaneous fat averaging 70 mm. in thickness over the chest and 162 mm. over the abdomen. The omentum is moderately adipose and the mesenteric vessels are embedded in lines of fatty tissue. The liver presents 4 cm. below the costal margin. A hernia, 3 cm. to the right of the umbilicus and 2 cm. above it, contains about 6 cm. of the transverse colon. Upon removing the sternum a fatty mass shot with smaller glandular masses and measuring 7.5 × 5 × 2 cm. is exposed, overlying the upper portion of the pericardium and occupying the site of the thymus. It weighs 40 gm. The heart weighs 605 gm. and is enveloped by a very adipose pericardium. The aorta shows the usual changes seen in atheroma of a non-specific type. The thyroid is rather large, globular and of a uniform reddish color; on section, the knife meets with considerable resistance and the colloid content is somewhat diminished.

Of the other organs, we shall mention only the following. The pancreas is superficially normal, but on section it proves to be congested, finely granular, soft and friable and somewhat invaded by fatty tissue. The suprarenals show postmortem softening and each contains a spheroidal mass of what appears to be cortical substance in the form of an adenoma; each mass measures about 1.5 cm. in diameter. These glands are soft and fleshy. The kidneys weigh 340 gm. together and show the typical changes of chronic vascular and glomerulonephritis. The ovaries are small and sclerotic.

The findings in the head require special consideration. Upon opening the calvarium, it is found to be from 0.5 to 1 cm. thick and rather compact. It presents a peculiar thickening of the inner table in the right parietal region in the form of thin, flat exostoses several centimeters long and one or two centimeters wide which are raised but a millimeter or so above the general level of the bone and form a roughly hand-shaped excrescence, the fingers splayed forward. There is a knob-like exostosis over the inner end of either petrous bone, near the attachment of the tentorium. The dura is thick and tough, but not remarkable, except for the presence of a nodular mass of firm, grayish tissue 0.5 cm. in diameter over the left tentorial margin.

The brain weighs 1115 gm., is shriveled and does not take up all the room allowed it by the cranial cavity, appearing to be definitely atrophic. The sulci are deep and wide and the convolutions nar-

row. An increased amount of cerebrospinal fluid escapes from the meninges. Over the right parietal convexity, in the region of the post-central sulcus, is a pyramidal depression, 4 × 2.5 × 2 cm. in size; section of this area reveals nothing but atrophy to account for the condition, but it underlies the exostoses already described. Careful examination of the hemispheres and basal ganglia reveals nothing of note; no definite areas of hemorrhage are encountered.

The pituitary body weighs 1 gm., and measures 17 mm. in length, 15 mm. in width and 7 mm. in thickness; the anterior lobe is large, yellow and tough and is asymmetrical, surrounding the pars posterior and extending higher on the right than on the left. The posterior lobe, while somewhat large, does not appear to be abnormal. The sella turcica is wide and shallow, measuring 10 mm. in length, 22 mm. in width and 5 mm. in depth. The pituitary must have bulged at the sides, when *in situ* to occupy such cramped quarters and probably expanded antero-posteriorly after it was removed. The sella turcica is somewhat discolored and reddened. Nothing abnormal is noted in the case of the mammillary bodies or tuber cinerium.

Microscopic Examination

Microscopic Diagnoses: Endothelioma of the dura with metastasis or double incidence, involving tentorium and stalk of the hypophysis. Slight adenomatous hyperplasia and marked focal sclerosis of the hypophysis. Sclerosis and colloid struma of thyroid. Rather extensive and active thymic rests. Adrenal hyperplasia and bilateral adenoma. Autodigestion of pancreas and mild acute pancreatitis with fatty invasion of the organ and fibrosis. Fibrosis of ovary with subacute oöphoritis.

Myocardial hypertrophy, focal fibrous myocarditis, coronary sclerosis. Chronic passive congestion of lung, liver and spleen. Pulmonary anthracosis and healed tuberculosis with hypostatic congestion. Perisplenitis and fibrous perihepatitis. Chronic cholangitis. Chronic vascular and intracapillary glomerulonephritis. Uterine adenoma (polyp) and chronic hyperplastic endometritis in a senile, fibrotic uterus. Senile atrophy of the brain.

Microscopic Appearance of the Tumors and Endocrine Glands

Tentorial Tumor. This is composed of slender, apparently fusiform cells with a rich cytoplasm which shows no demonstrable fibrillae with Van Gieson's, Bielschowsky's or Mallòry's phosphotungstic acid hematoxylin stains. Mitotic figures are present in moderate numbers, usually occurring in small cells that lie between the tumor cells. The arrangement of the growth is in sheets and concentric whorls, as shown in Fig. 2. The sheet-like leashes of cells that one sees running through the tumor have larger nuclei than those in the whorls. Tumor giant cells may be found occasionally. Between many of the cell masses one finds deposits of dense collagenous tissue which stain selectively with appropriate dyes. Occasionally a corpus amylaceum is encountered and calcification of the collagen masses is sometimes observed, as well as hyaline degeneration thereof. The tumor has infiltrated the dura to a moderate extent.

Infundibular Tumor. This was discovered only after microscopic examination was made. It lies close to the stalk of the hypophysis in the dural sheath and is almost identical in its appearance with the tentorial growth, but tends to grow in wider sheets (Fig. 3). It infiltrates the dura and has almost penetrated it in places (Fig. 4). Small islands of tumor cells are seen in the dural spaces at some distance from the main growth, indicating metastasis. The masses of collagen and the psammomatous bodies are not as numerous in this tumor as in that on the tentorium, but mitotic figures are rather more abundant.

Hypophysis. The gland shows a definite hyperplasia, amounting almost to a struma and there is much sclerosis near the site of the tumor in the anterior lobe (Fig. 5). The appearance of the gland does not differ very much from that noted in pregnancy, but this woman was neither pregnant nor had she borne children. All the cells of the anterior lobe contain neutral fat in coarse drops and masses in the chromophil and in very fine dust-like granules in the chromophobe cells. This substance stains yellow to orange with Sudan III, rose-pink with Nile blue sulphate and from brownish yellow to black with osmic acid. Fig. 6 was photographed from a frozen section stained with Sudan III. There is so much black and brownish material in the Bensley stain, that little of the cytoplasmic

details can be made out. There is definite sclerosis only in that portion of the gland that abuts on the tumor. The pars intermedia contains the usual colloid masses in its acini; but some of them have increased until they constitute retention cysts. The pars posterior does not seem very abnormal, as compared with a normal control.

Thyroid. As seen in Fig. 7, this is rather less rich in colloid than it should be, as that substance is thinned out, vacuolated and scalloped at the periphery, although the acini are larger than normal. The connective tissue is increased in amount. No embryonal acini are found.

Parathyroid. This is very much congested and contains some retention cysts filled with colloid material, but it is not abnormal for a woman of advanced age.

Thymus. Although it consists chiefly of fatty tissue, there are numerous small islands of thymic tissue which assume respectable proportions under the microscope and show Hassal's corpuscles and "thymic reticulum cells," as well as rather sparsely distributed lymphocytes. There is no necrosis of the Hassal's bodies which are apparently as well preserved as they would be in a child of a few years of age.

Suprarenal Glands. Both of these are hyperplastic and the central softening, noted at necropsy, is represented by poor staining and dissolution of the tissue of the medulla. The tumors contained in these glands are composed of cortical cells very lawlessly arranged (Fig. 8); they may be grouped into glandular complexes, or dissociated. There is no suggestion of hypernephroma. Areas of hemorrhage and groups of pigmented phagocytes are seen and lymphocytes have infiltrated the gland rather abundantly in places.

Pancreas. Unfortunately this has undergone rather extensive postmortem autodigestion and shows much necrotic parenchyma. As there are areas of polymorphonuclear infiltration, one wonders whether it was a terminal infiltration due to this process, or whether there was an early inflammatory reaction in some way connected with the cholecystitis and cholangitis. Where the pancreatic tissue has escaped this process, the islets and the parenchyma are apparently normal. There has been considerable invasion of the organ by adipose tissue and fibrous connective tissue and the fat cells have been affected by the pancreatic lipase and appear as they do in "fat necrosis."

Ovary. There is marked senile sclerosis, many corpora hyalina, a number of follicular cysts and, at one pole, a collection of endothelial phagocytes and some epithelial rests resembling kidney tubules.

Other Organs. Microscopic examination of the other organs does not show anything that adds greatly to the value of this paper and the diagnoses have been listed elsewhere. The skeletal fat is not abnormal, nor do the nerves it contains show any striking changes in appearance. The brain fails to show any hemorrhages and the only demonstrable lesions are porosity and gliosis, such as one would expect to find in senile atrophy. There is a generalized lymphocytic infiltration of a very insignificant degree and the small glia cells are notably increased in number.

Discussion

We could discuss this case with a great deal more assurance had we been able to study it longer while the patient was alive. The meagerness of the history and the impossibility of observing her over an extended period of time make it difficult to correlate the symptoms that may have been present with our pathologic findings. Our patient showed the following signs and symptoms of adiposis dolorosa: huge fat masses on the body and extremities, but no adiposity of the head, forearms, hands, legs or feet; a history of recent epileptiform convulsions and at least a transient psychosis (uremia should be considered here); subnormal temperature; asthenia; ulcers on both shins (possibly varicose); but no history of pain or tenderness over the fat masses or nerve trunks. Had she lived longer and retained consciousness, she might have been able to enlighten us on this point and she may have had such symptoms in the past without her friends hearing of them.

The necropsy findings coincide very accurately with those in undoubted cases of adiposis dolorosa. The very definite lesions in practically all the endocrine glands are striking: pituitary sclerosis and hyperplasia, with a tumor; sclerosis and changes in the colloid content of the thyroid; persistent and well preserved thymic rests; adenoma of both suprarenals, with hyperplasia; ovarian sclerosis and atrophy; and definite, though slight, changes in the pancreas. Besides these, we see changes in the cranial bones, with exostoses and definite cerebral atrophy, with some generalized thickening of the dura.

How is one to interpret these lesions? We may rule out the ovarian sclerosis on the obvious basis of senility, but we do not know how long it was present. We may ignore the pancreatic changes as the result of postmortem autolysis and senility; we may disregard the thickening of the dura, skull and meninges and the cerebral atrophy, as representing racial and senile changes. We cannot, however, dismiss the pluriglandular pathologic condition of the endocrine system in general.

Perhaps the most striking thing about the case is the presence of two atypical dural endotheliomas, one involving the stalk of the hypophysis. While this tumor is usually essentially benign and produces much reticulum, our tumors were evidently malignant (as judged by the primitive type cell, the presence of mitotic figures, the infiltration and the possible metastasis) and they produced no reticular or collagenous matrix. We cannot say that the tiny tumor of the hypophyseal stalk was metastatic from the tentorial tumor, but that is the natural inference. If that were true, how could one explain the long-standing adiposity and asthenia of the patient? Possibly by supposing that the over-acting hypophysis long antedated and, later, predisposed toward tumor growth by stimulating it as it did the endocrine organs elsewhere. Be that as it may, the hypophysis seems to present more marked changes than do the other endocrine organs in this case.

Dercum [1] was inclined to ascribe his syndrome to a lesion of the thyroid. Cushing [3] believes that many cases reported as adiposis dolorosa "are actually examples of disturbed metabolism secondary to diseases of the ductless glands." He does not consider this disorder to be a clinical entity, maintaining that it begins as a disturbance of the pituitary body affecting the thyroid secondarily. In later papers Dercum seems to be of the same opinion. Cushing reports an interesting case with all the typical features of Dercum's disease, in which a necropsy revealed no change in the pituitary, but generalized and extreme cerebral atrophy; he proposes that such cases be termed "adiposis dolorosa cerebralis." Price [4] found changes in the pituitary gland in both of his cases, but believes that those in the thyroid are of equal importance. What of the suprarenals? They, too, have shown strikingly constant pathologic changes in this disease.

The protocols of the sixteen cases in the literature and of ours,

point out quite clearly that the findings are not compatible with the assumption that adiposis dolorosa is a clinical entity and that Cushing is correct. The hypophysis has shown nothing at all, slight lymphocytic infiltration, adenomatous hyperplasia and sclerosis or it has been the site of tumors. The thyroid picture varies from nothing pathologic to sclerosis or even colloid struma. The thymus has not been mentioned hitherto, so that our case is apparently unique in this respect. The suprarenals have shown adenomatous hyperplasia pretty constantly. The gonads have been sclerotic, or "defective," which is compatible with the advanced age of most of the patients described and may not be very important unless constantly found in younger subjects. The fact remains, however, that something is usually wrong in most, if not in all of the endocrine glands in these cases and we must find out why this is so before we can declare the matter settled. Is not adiposis dolorosa merely the typical picture of a majority of a certain group of cases in which the endocrine system is involved and the fat metabolism interfered with?

Conclusions

The pituitary body is apparently at fault in this case, as in most of those that have come to necropsy. The changes in the other endocrine glands seem to follow as a result of this. It seems reasonable to suppose that sclerosis of the thyroid, persistence of thymic tissue in a patient of 60 years and adenomas of the suprarenals could be explained on a basis of overstimulation by the hypophysis. One hesitates at speculating too broadly on this subject for fear of allowing one's conclusions to become purely metaphysical and unwarranted by the facts at hand. It is justifiable, however, to ascribe the pathologic findings in this case to a profound disturbance in the endocrine system, probably arising as a result of one of the lesions found in the hypophysis cerebri.

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DESCRIPTION OF PLATES

PLATES 47–50

FIG. 1. Photograph of the patient after necropsy. Note the comparatively normal size of the legs and forearms, the moderate adiposity of the head and the great masses of fat on the trunk, arms and thighs. The ulcers on the ankles are also visible.

FIG. 2. Section of the tentorial tumor. A mass of hyaline colloid is at the center. Hematoxylin and eosin stain. x 300.

FIG. 3. Section of tumor on hypophyseal stalk. A corpus amylaceum is clearly shown. Note whorls. Phosphotungstic acid hematoxylin stain. x 300.

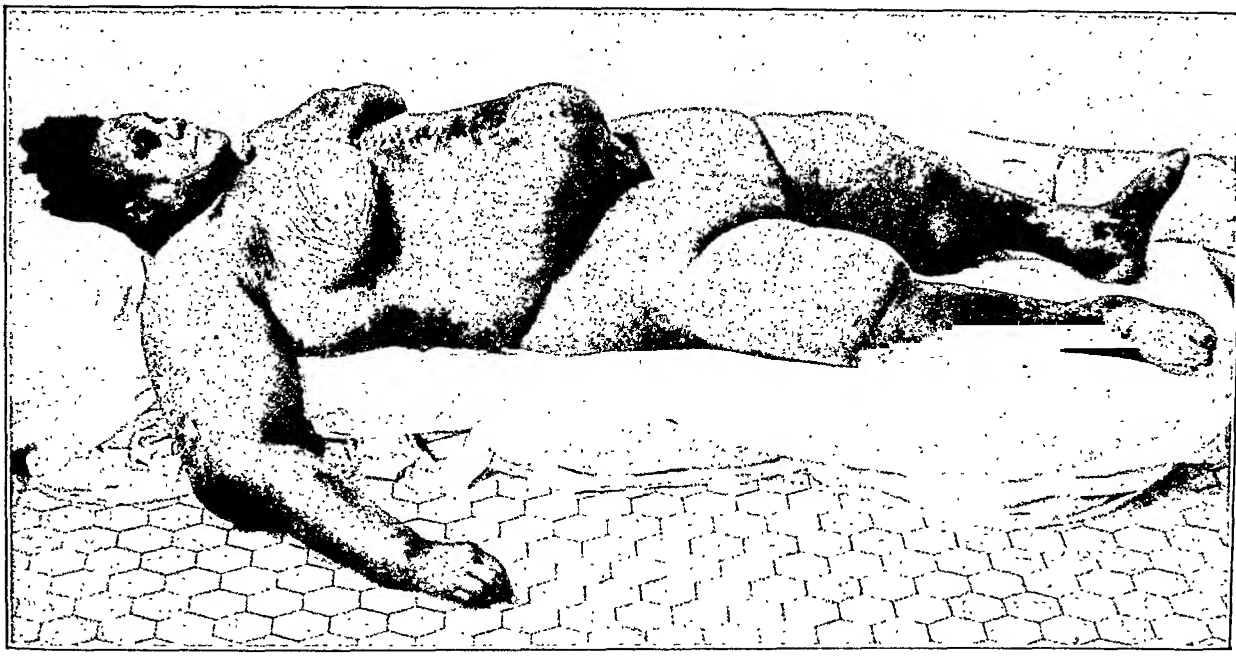
FIG. 4. Tumor infiltrating the dura over the hypophyseal stalk. Hematoxylin and eosin stain. x 300.

FIG. 5. Section from a distinctly sclerotic portion of the hypophysis. Hematoxylin and eosin stain. x 300.

FIG. 6. Frozen section of hypophysis stained with Sudan III, to show fat droplets (black in the photomicrograph). x 300.

FIG. 7. Section of thyroid, showing vacuolization and erosion of colloid and marked sclerosis of supporting framework of gland. Hematoxylin and eosin stain. x 300.

FIG. 8. Section through an adenoma of the suprarenal, showing the composition of the tumors in this case. Hematoxylin and eosin stain. x 300.



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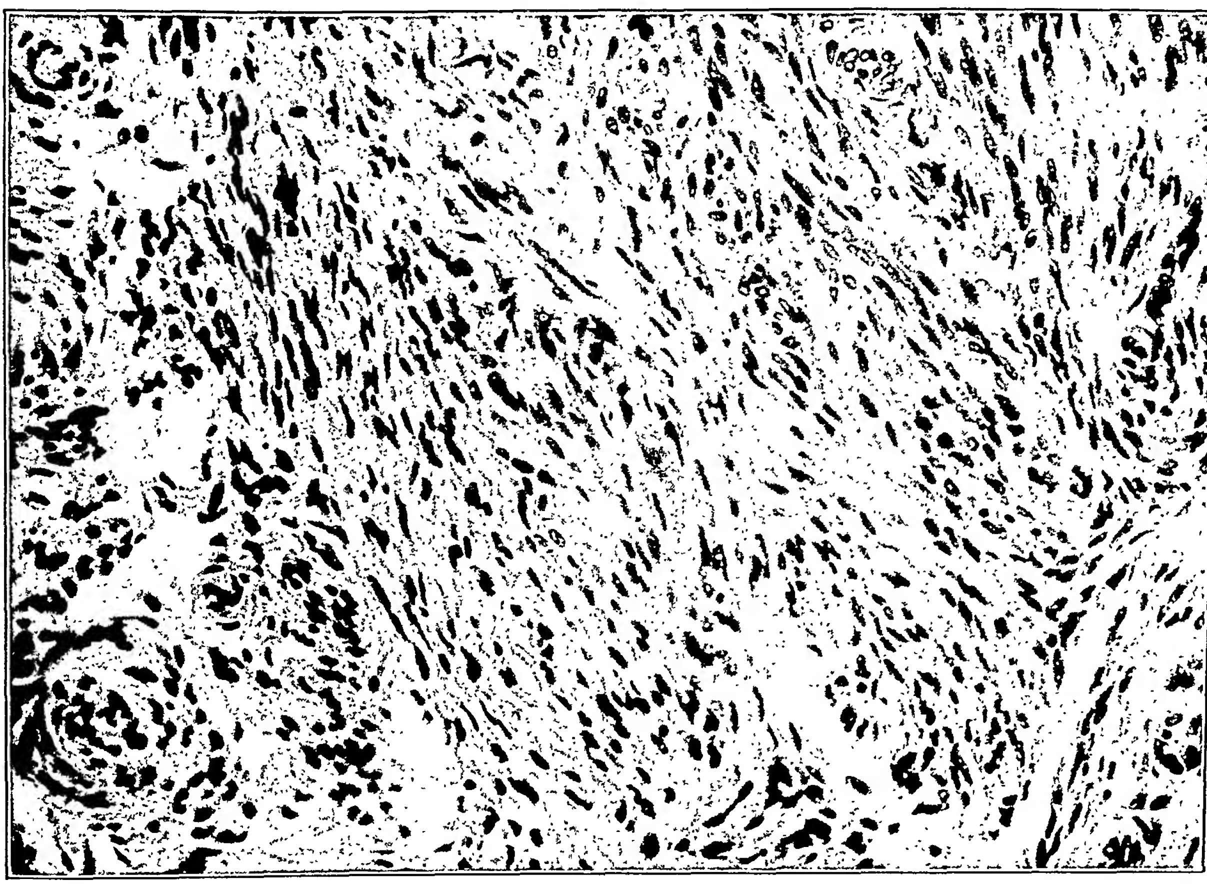

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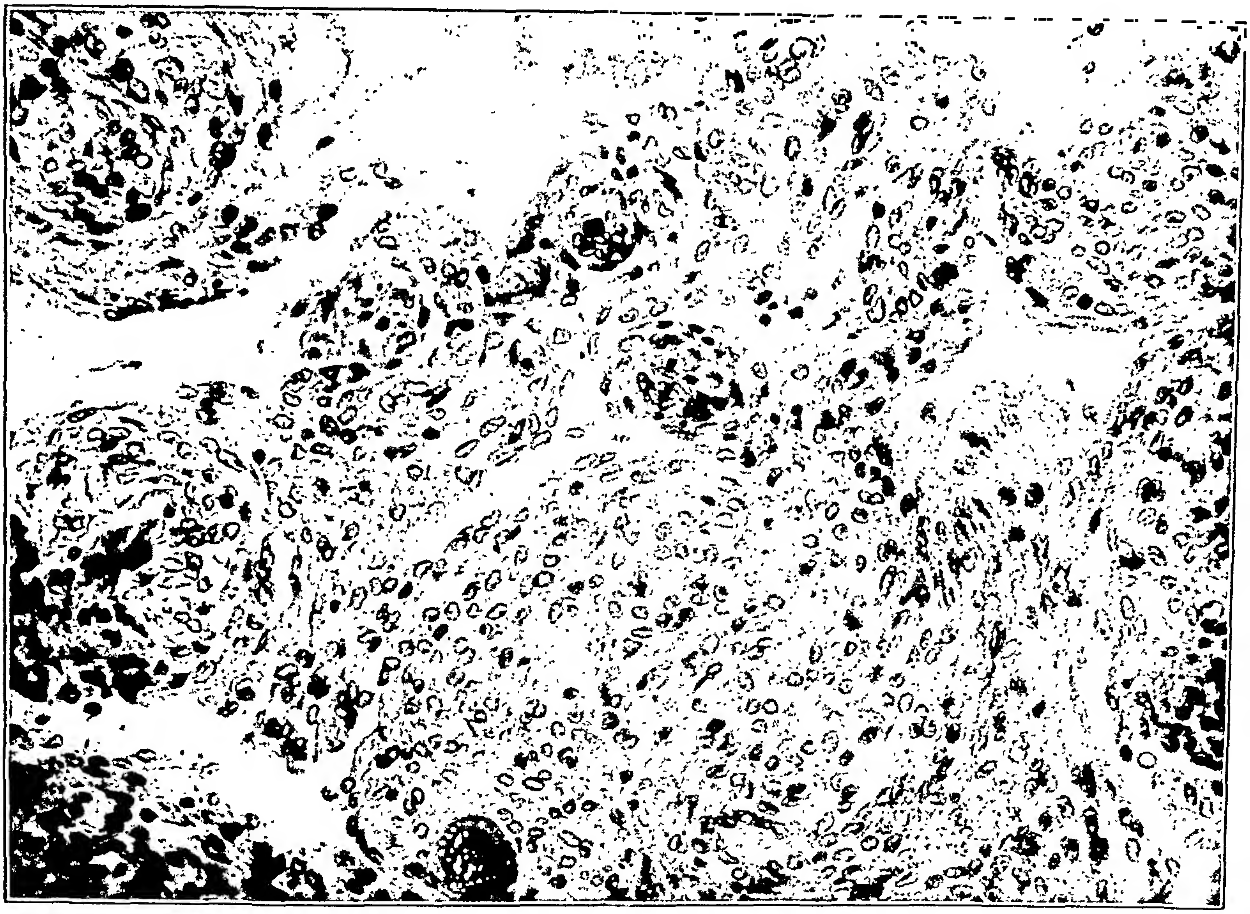

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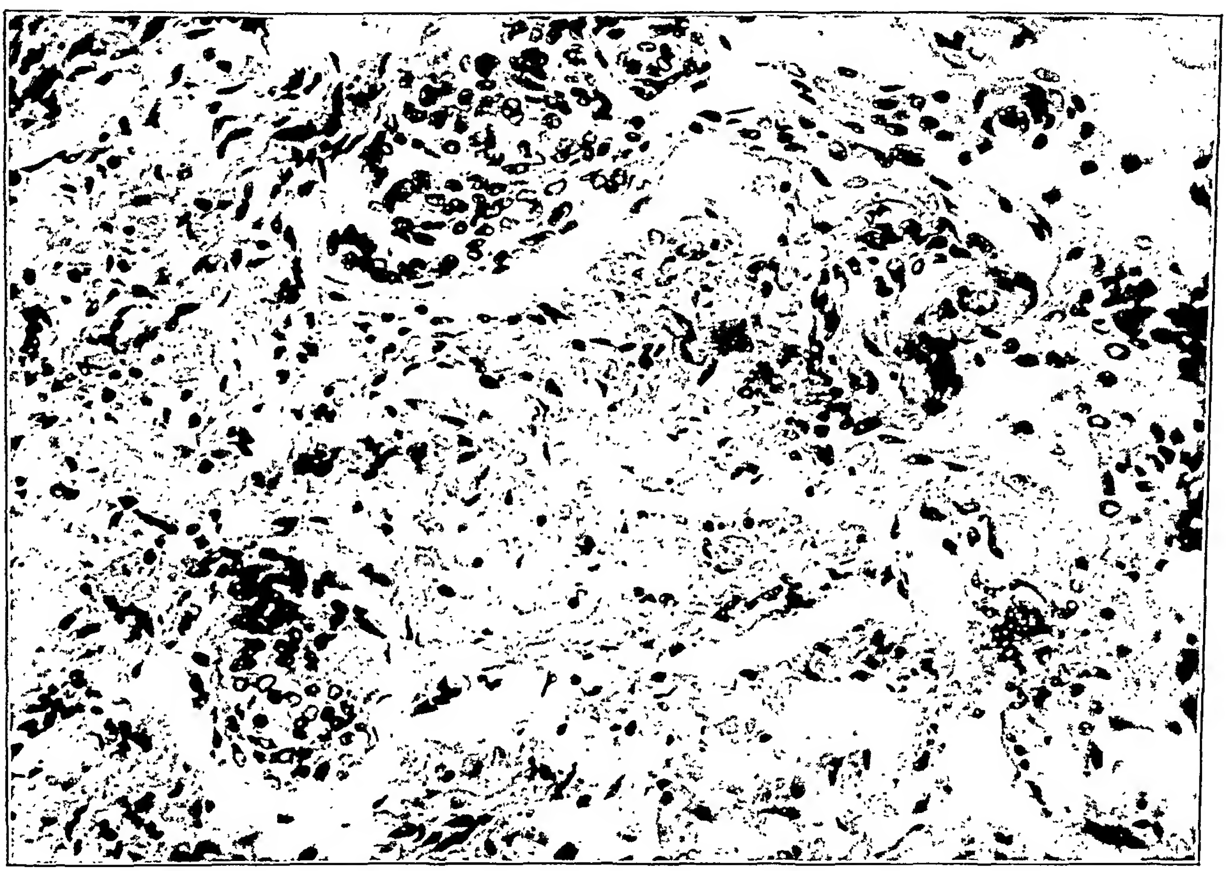

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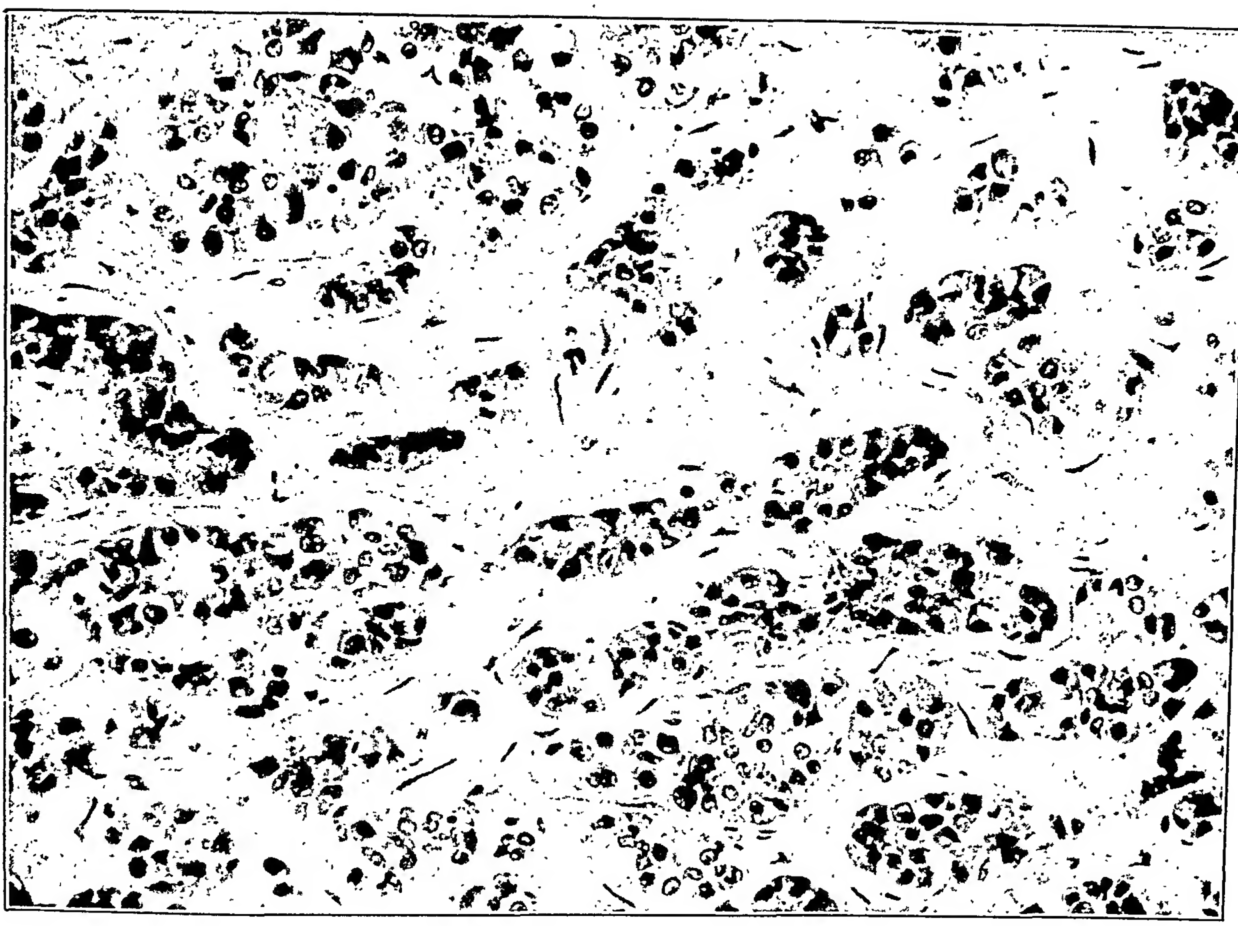

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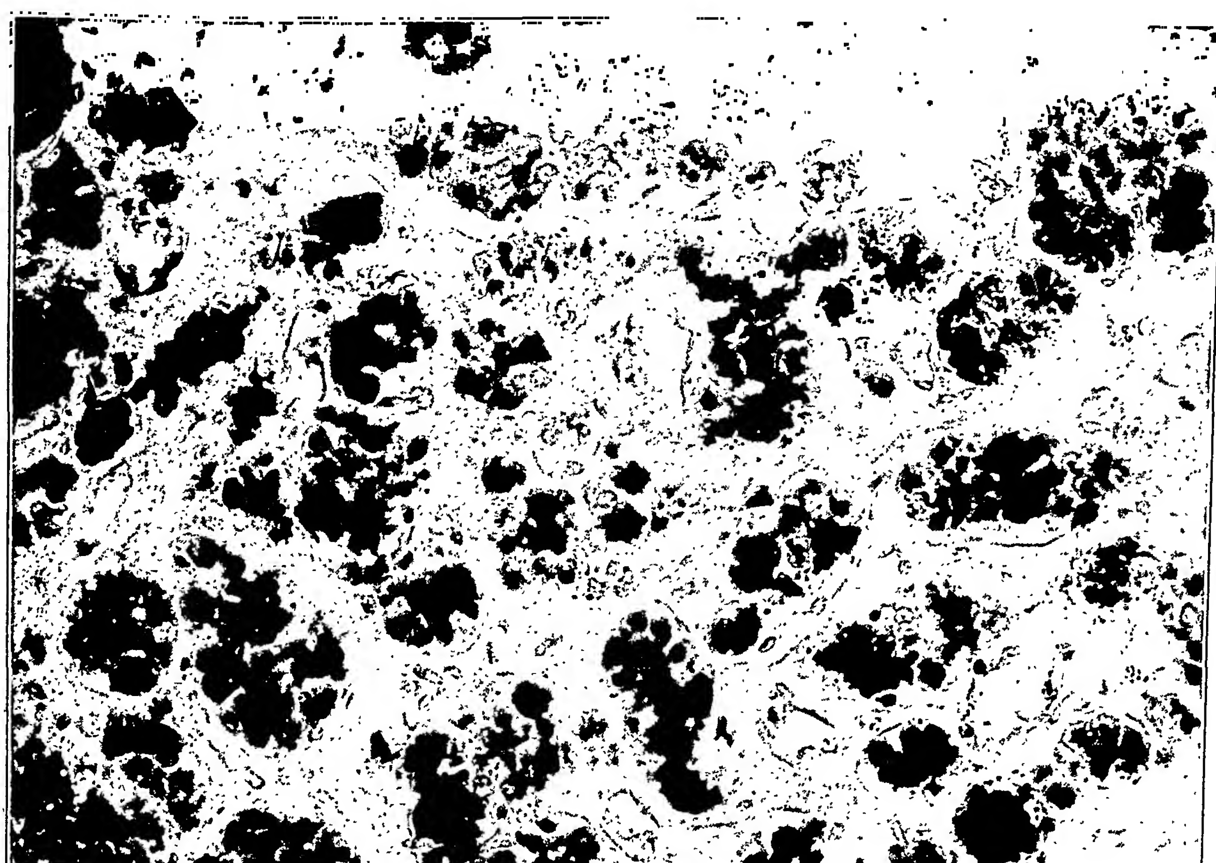

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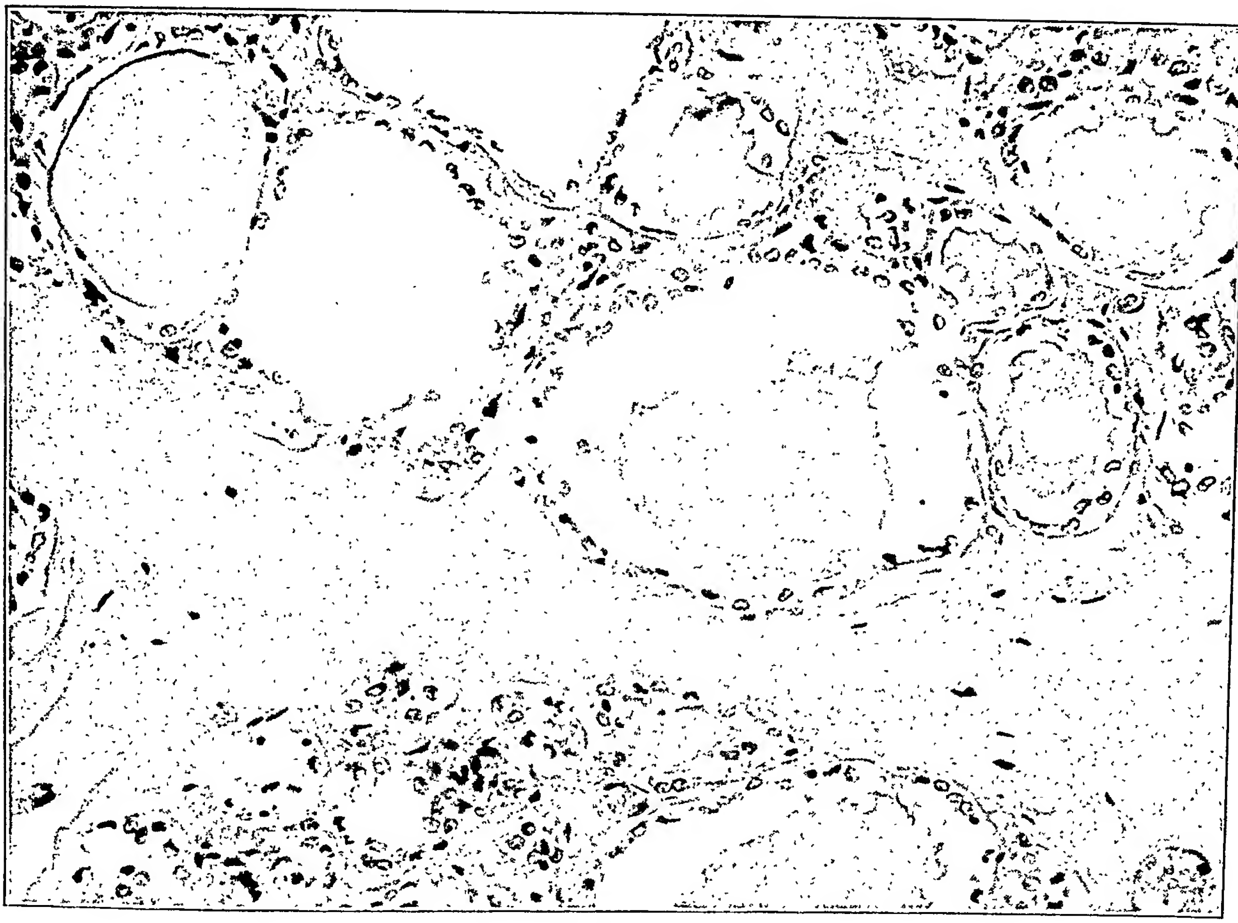

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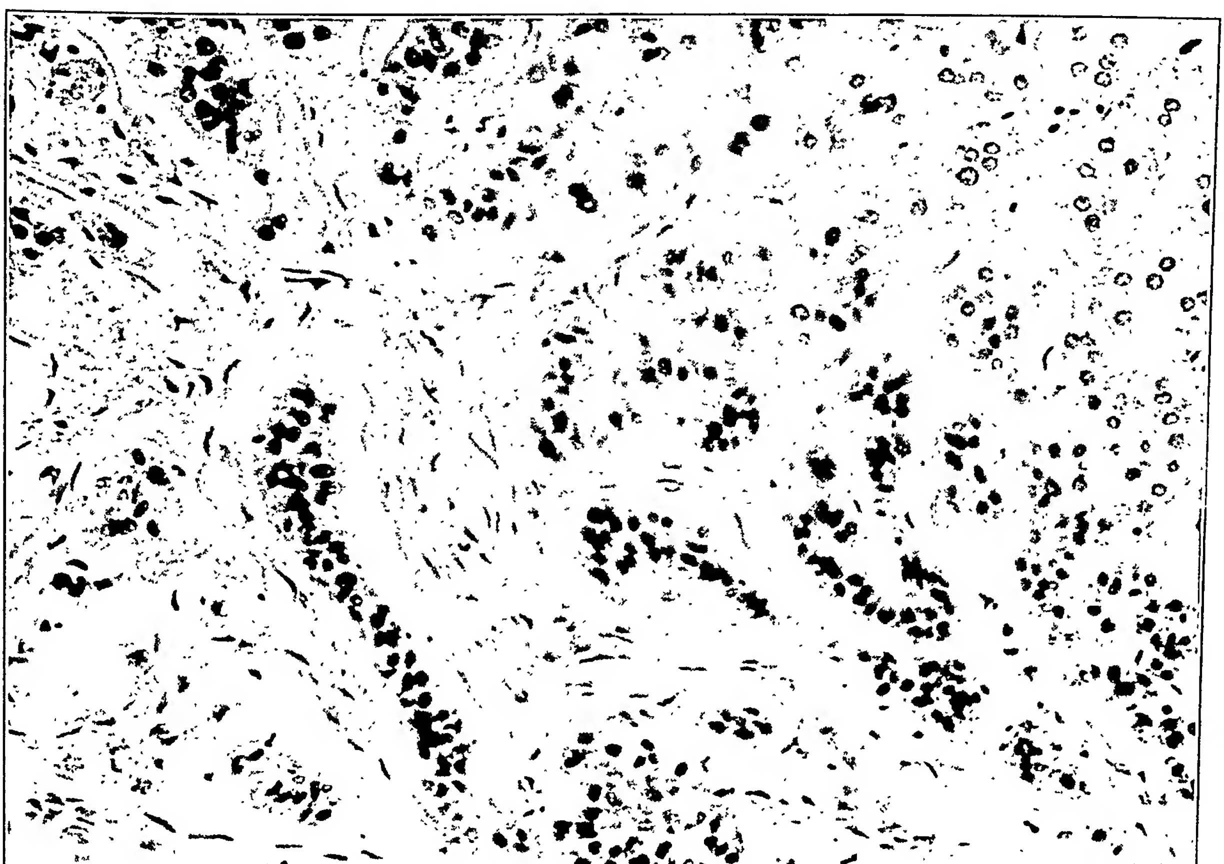

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THE HISTOPATHOLOGY OF THE SUBCUTANEOUS LESIONS IN TULAREMIA IN MAN *

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Introduction

The question of tularemia was brought acutely to our notice in December 1925, when Maclachlan, Fetter and Cratty [1] reported the first two cases from Pennsylvania. Since then, a third case has appeared in the Mercy Hospital on the surgical service of Dr. Weil, and within a few weeks three others in different hospitals in Pittsburgh.

Our interest in the disease was stimulated by the study of the tissue reactions in subcutaneous lesions obtained from Dr. Weil's patient by biopsy. These tissues form the basis of the present paper. No attempt will be made to cover the history of tularemia which Francis [2] has already done, nor to review its growing literature except that part pertaining to the pathology of the disease.

Case Report

Mrs. M. H., aged 48, was admitted to the hospital on Jan. 30, 1926, complaining of pain and swelling in the right arm and axilla. The relevant points in her history are those related to the chief complaint. She was employed in a butcher's shop and on Nov. 19, 1925, while at work dressing rabbits for sale, she scratched her right thumb on a splintered rabbit bone. The animals were of the common wild variety and had been shipped from Kentucky. The wound was slight and was given no attention. Three days later the thumb became painful, and the patient had two severe chills with nausea. When seen by a physician the next morning, she had a fever of 105 F and there were "red streaks" observed on the forearm as far as the elbow.

After remaining in bed with wet dressings for a few days, the thumb was incised and pus was found. Two weeks later the primary

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lesion again required incision, and at about this time inflammatory nodules appeared on the flexor surface of the forearm, following the distribution of the lymphatics. These appeared after chills, fever and subjective symptoms such as had accompanied the infection in the thumb. Some of these lesions required drainage. The entire illness was characterized by weakness and malaise. As there was no real improvement she was sent to the hospital, coming in about ten weeks after injury.

At the time of admission, she appeared to be in fair general condition, the peculiar lesions of the right arm being the only important finding. The thumb showed a recently healed wound. At the wrist and elbow and in the axilla were small sinuses discharging a seropurulent fluid from chronic indolent inflammatory foci. An indurated subcutaneous nodule about 1.0 cm. in diameter was noted near the midpoint of the forearm, where the overlying skin was dull purplish red. A fluctuating mass could be felt in the axilla, but here the skin showed no change as this mass was more deeply placed. The entire group of nodules followed the lymphatic distribution on the flexor surface. Later (about March 9), the lymphatic involvement became unmistakable. The superficial lymphatics could be palpated through the skin of the forearm as a beaded cord-like structure.

Further investigation at that time revealed little of a positive nature. Her temperature was 97.6 F. A blood count showed 12,000 leucocytes with 63 per cent polymorphonuclear neutrophiles. The blood pressure was unusually low, 86 systolic and 60 diastolic, corresponding with the depressed state. X-ray examination of the thumb showed no bone involvement. The blood Wassermann was negative.

On Feb. 1, 1926, the nodule on the forearm and the axillary swelling were drained. Both contained a creamy light greenish yellow pus which showed no organisms by the usual stains, but contained many pus cells. Cultures on ordinary media showed no growth from the nodule on the forearm, though a *Staphylococcus albus* was recovered from the axillary lesion. A biopsy was done on the nodule on the forearm at the time of incising it (first biopsy).

No special reaction followed this operation. The patient was not bedfast, but it was thought better to confine her to bed owing to her peculiar weakness and malaise. On Feb. 7, 1926, a temperature of 99.8 F was recorded with chill, nausea and increased subjective symptoms. The upper arm became tender and painful over the

biceps region and in a few days two definite subcutaneous nodules appeared. A blood count at this time showed a slight decrease in the total leucocytes and in the polymorphonuclear neutrophiles as compared to the previous count. On Feb. 11, 1926, she developed another rather severe chill and slight rise in temperature. This reaction was likewise accompanied by increased malaise and nausea. It was the sixth or seventh exacerbation and as before a new lesion soon developed, this time in the axilla near the two earlier ones. On Feb. 24, 1926, the two subcutaneous nodules on the upper arm were excised intact with the skin and subcutaneous tissue (second biopsy). Both biopsy sites healed readily; the second by primary union, while the first cleared up more readily than the axillary lesion from which no tissue was excised. However, the secondary infection in the axillary focus explained the delay in healing.

On February 27, the patient complained of pain and tenderness in the right lumbar region. Examination revealed a definite tender mass here and a similar but less tender one on the left side. These have slowly regressed since that time. (One of Hodges' [3] patients developed nodules in the thoracic region of the back.) These lesions appeared after a chill which was accompanied by flashes of heat over the body, particularly felt in the right arm and in the legs. Sattler's [4] case had the same subjective symptoms.

Tularemia was considered as a diagnosis from the history and clinical findings. In view of the negative bacteriologic results and especially after obtaining an unusual microscopic picture in the first biopsy, a sample of the patient's serum was sent to Dr. Edward Francis, Hygienic Laboratory, Washington, D. C. On Feb. 10, 1926, he reported the agglutination of *Bacterium tularense* by this serum in dilutions of 1:10, 1:20, 1:40, 1:80 and 1:160, but not higher; which, he stated, confirmed our diagnosis. On Feb. 19, 1926, he reported the same titer on a second sample taken ten days after the first. A third sample, taken March 20, 1926, again showed the same titer.

Further studies leading to a determination of the causative agent were carried out on the tissue, but like the bacteriologic analyses were chiefly valuable as negative evidence. Sections stained by the Gram-Weigert and Ziehl-Neelson methods revealed no bacteria of any type. Twort's [5] light green-neutral red mixture was then tried in an attempt to stain *Bacterium tularense* in the tissue. This also

gave negative results. Animal inoculation was not done. The patient is slowly convalescing, and no further operative interference has been indicated.

Pathologic Report

The first specimen obtained consisted of a wedge-shaped piece of tissue from the indolent-looking purplish red inflammatory nodule on the flexor surface of the right forearm. When the nodule was incised a few drops of creamy, greenish yellow pus flowed out. The biopsy material was then taken and dropped immediately into Zenker's fluid. It represented a quadrant of the complete lesion with the overlying skin.

The second specimen was removed from the same aspect of the upper arm. It represented a more recently developed lesion and consisted of two small subcutaneous nodules lying close together and easily felt through the skin. These were excised widely, together with the overlying skin. The material was fixed in Zenker's fluid and sliced after it had hardened slightly. The nodules were small abscess-like structures measuring respectively 0.6 and 0.3 cm. in diameter, located in the subcutaneous tissue. They showed dull yellow walls and contained a drop or two of creamy light greenish yellow pus. There was no gross evidence of hemorrhage or of congestion.

The second biopsy furnished better material, since the lesion thus obtained was complete and in an earlier stage of development. It will therefore form the basis for the microscopic description, and additional features illustrated by the more advanced lesion will be described in the appropriate place.

Histopathologic Report

The sections show circumscribed inflammatory nodules lying below the dermis and in the fatty areolar tissue, rather than in a lymph node. Spaces representing adipose tissue cells are noted throughout the nodules. The process apparently originated in lymphatics. The inflammatory reaction is somewhat unusual.

A typical inflammatory nodule shows a necrotic center, in which granular débris is packed with polymorphonuclear leucocytes and degenerating mononuclear cells of the endothelial type. Sometimes a degenerating giant cell lies free in the necrotic material. Surround-

ing this and dividing it from the living tissue is a narrow zone (not more than five or six cells deep), of granular, partly degenerated endothelial leucocytes which lie in a delicate, irregular meshwork of fibrin. Just beyond this is found a clearly marked zone of newly formed tissue having all the essentials of granulation tissue, though with certain differences affecting both the fibroblast and the capillary elements.

The capillaries in this zone of newly formed tissue are few and small, with narrow lumina as a result of hyperplasia of the endothelium. The reactions in capillaries and small vessels are important and will be referred to again. The fibroblasts are numerous, large and closely packed. They are plump enough to resemble flattened endothelial cells, and their radial arrangement suggests that commonly seen in caseous tuberculous lesions. Phosphotungstic acid hematoxylin and Mallory's anilin blue stains demonstrate fibroglia fibrils in these cells as well as areas of early collagen formation in parts of the fibroblastic zone, thus identifying the predominating cells of this zone as fibroblasts.

Inflammatory cells of all types, including many endothelial phagocytes, are scattered throughout this zone in about equal numbers without forming definite collections. Further, a striking and unusual finding is encountered in the presence of many large giant cells much like the Langhans type. These are practically confined to the fibroblastic zone.

The outermost zone of the nodule is made up of a dense chronic inflammatory cell exudate in which lymphocytes and plasma cells predominate. In the areolar tissue beyond this, the same cell types form dense, perivascular collections. Occasionally a small vessel shows definite perivascular and medial inflammation. Some adipose tissue cells are partly surrounded by endothelial cells, with early giant cell formation. Capillaries are somewhat more numerous in this zone than in the inner fibroblastic one; but in both locations, they show the remarkable proliferative reaction of the endothelial linings briefly referred to above. The usual wide, thin-walled capillaries of granulation tissue are not conspicuous. Instead, most of the capillaries are rather narrow. Endothelial hyperplasia is most marked in the sections from the larger, more advanced lesions of the first biopsy. In any section many examples of the reaction can be found, usually with one or two showing complete obliteration of the

lumen. In some, mitotic figures appear in the endothelial cells, indicating marked proliferative activity. The capillaries all contain many polymorphonuclear leucocytes in and about them. Those with piled up endothelial linings show polymorphonuclear leucocytes lying between the cells of the thickened wall.

Finally, and further contributing to the granulomatous character of the lesion, there is noted the development of small secondary necrotic foci in the wall about the large necrotic center. These are sometimes merely collections of endothelial cells with a small giant cell. Again they are more advanced, showing necrotic centers filled with polymorphonuclear leucocytes. These centers are surrounded by a few endothelial cells beyond which lies a fibroblastic zone. In a word, the secondary foci are small replicas of the large lesion. These can often be found related to obliterated capillaries. The heavier fibrous bands of the reticular tissue appear to have a slight limiting effect on the extension of the lesion. At the same time they show fibroblastic proliferation as an evidence of an inflammatory reaction in which they partook to some degree.

Through the kindness of Dr. A. J. Bruecken of the St. Francis Hospital and Dr. J. W. McMeans of the St. Margaret Memorial Hospital, we have been permitted to study material from two of the three other Pittsburgh cases. In each the diagnosis was verified by agglutination tests, the titers running up to 1:320 and 1:640 respectively (Francis). Both specimens consisted of axillary lymph nodes. The histopathologic findings are identical in all essentials with those found by us in the subcutaneous nodules.

Discussion

We have been interested in presenting a description of the subcutaneous lesions in human tularemia, since no detailed study of them is available in the literature. Sattler,[4] in 1915, reported the excision of a small nodule from the conjunctival sac which Woolley described as "a granuloma without giant cells apparently occurring in a lymph gland." Ohara, quoted by Francis and Moore,[6] reported the pathology in an axillary lymph gland as follows: "Histologic examination of the lymph gland excised in the human experimental case showed round cell infiltration, dilatation of the blood vessels, extravasation of blood, small pus cavities, caseation and giant cells." The only

other description of human lesions of any type is that of Verbrycke[7] who reported a fatal case with necropsy. Francis[8] also examined this material and wrote of it in part as follows: "Microscopic sections of spleen and liver showed the small areas of focal necrosis typical of the disease in guinea-pigs and rabbits . . . the enlarged peribronchial lymph glands and nodules found in the lung substance showed the areas of focal necrosis; there were giant cells in the peribronchial lymph nodes. No tubercle bacilli could be demonstrated in lung, spleen or liver." The lymph nodes from the other recent cases in Pittsburgh, which we have cited briefly above, complete the list of human lesions which have come to microscopic examination.

The clinical picture of our case corresponded well with the cases of this type in the literature. It particularly resembled that of Hodges.[3] The gross appearance of our specimens was also typical of the human lesions of Hodges,[3] Verbrycke,[7] Shelton,[9] Sattler[4] and others, as well as of the two more recent Pittsburgh cases.

The general resemblance to the described animal lesion was evident. Woolley,[10] in 1915, published the first microscopic study of tularemic lesions, made on the experimental animals of Wherry and Lamb[11] (1914). He described the reaction as a primary necrosis, with suppuration only in the skin lesions. He found no evidence of any marked proliferation of endothelial cells. The earliest change he believed to be degeneration of a focal area, with beginning karyorrhexis. At this stage he found a few lymphocytes, and noted that the polymorphonuclear leucocytes appeared after degeneration of tissue had occurred, adding that were there more endothelial proliferation, the lesions might resemble those seen in the liver in typhoid fever. Councilman and Strong[12] (1921), described clearly the pathologic histology in the acutely fatal lesions of animals. Their report is of the greatest interest in this connection, since they described the same basic reactions in the animal tissues that we found in the human. They stressed endothelial hyperplasia and the mononuclear character of the early cellular exudate, noting that the polymorphonuclear leucocytic response was entirely secondary. They were able to stain the *Bacterium tularense* in the liver cells, and also in the vascular endothelium. In summing up, they stated that "The essential lesion is infection of the endothelial cells, general but more marked in the vessels of certain organs." Ledingham and Fraser[13] (1924) published the only other microscopic study of

experimental lesions. They emphasized the demonstration of *Bacterium tularense* in the tissues; otherwise their descriptions coincided with Woolley's.[10] They failed to note the vascular changes and endothelial cell exudate described by Councilman and Strong.[12]

We feel it is evident that the descriptions available in the literature (with the exception of that of Councilman and Strong [12]), are not entirely clear and that they offer no basis for an explanation of the peculiar necrotic lesions of tularemia. It is also quite evident that the inflammatory reaction we have described is a granuloma. This character does not seem to appear in animal lesions which are far more acute than those of man. The chronic clinical course typical of the disease in man is suggestive of a granulomatous type of reaction.

In view of this granulomatous character, the explanation of the human lesions we have studied becomes fairly simple. They are in some respects similar to the reactions in typhoid, syphilis, actinomycosis and blastomycosis, though at first glance the suggestion of a caseous tuberculous lesion is very strong. The infection is evidently blood-borne in animals, while in the usual human case its manifestations suggest a local lymphatic spread, accompanied by a marked toxemia. The possibility of a temporary and even recurrent bacteriemia in the human must also be considered. The type of injury is apparently mild, though more active than that of tuberculosis. The reaction tends toward healing, and this process is greatly facilitated by drainage.

To confine the discussion to the subcutaneous lesion, the first reaction is one leading to endothelial proliferation in capillaries with the local production of wandering cells, resulting in endothelial collections and giant cell formation about the bacteria. Excessive capillary reaction of this type tends to narrowing and even obliteration of the capillaries. Mitoses indicate that this process is quite active. As a result of these intracapillary changes, necrosis takes place in the areas rendered anemic. The action of bacterial toxins must also be considered, but a capillary obstruction best explains the sudden and relatively extensive necrosis in which degenerating endothelial and giant cells can still be made out.

The presence of necrosis stimulates a marked polymorphonuclear leucocytic exudate which infiltrates the necrotic area and plays a part in bringing about softening. The fixed tissue response about the

necrosis consists of a cellular granulation tissue, in which are many giant cells. The endothelial proliferation continues and, with further obliteration of capillaries, small secondary necrotic foci are formed in the granulation tissue wall about the primary area of necrosis. The numerous giant cells in the fibroblastic zone indicate the sites of beginning secondary necrotic foci.

In the meantime, polymorphonuclear leucocytes continue to migrate to the necrotic area. They may be seen filling the capillaries, emerging from them and lying in their vicinity. Even from the vessels with piled-up linings, the polymorphonuclear leucocytes still continue to wander out into the tissues. We find sometimes that a completely obliterated capillary, a mere whorl of endothelial cells, shows many polymorphonuclear leucocytes in the interstices between the endothelial cells obstructing the lumen.

These changes are interesting as tending to show that local capillary endothelium may undergo great activity in the production of wandering endothelial cells, as has been claimed by Mallory,[14] Mallory and Medlar,[15] Foot,[16] Permar[17] and others. The presence of mitotic figures in the cells of the capillary walls is also in favor of this theory. It must be admitted that many of the wandering endothelial cells may have originated in the more commonly active endothelial beds; but the capillaries do not contain free mononuclear phagocytes in even appreciable numbers, though this cell forms a good part of the local exudate.

The perivascular lymphoid and plasma cell infiltration found at the periphery of the lesion is characteristic of any chronic inflammatory process, and notably of the granulomas. The involvement of the walls of small vessels is apparently by extension from the perivascular lymphatics. The reaction about fat tissue cells is the result of injury incident to their inclusion in the involved area.

Conclusions

1. *The histopathology of the subcutaneous lesion in human* tularemia is described in detail.

2. The lesion presents the microscopic characteristics of a granuloma.

3. The tissue reaction may be summarized as follows: (*a*) primary massing of endothelial cells with giant cell formation; (*b*) endothelial

hyperplasia with obliteration of capillaries; (*c*) necrosis with polymorphonuclear leucocytic infiltration and liquefaction; (*d*) development of small secondary lesions which pass through the same stages and tend to fuse with the primary one; and (*e*) delayed healing by organization.

The authors wish to express their appreciation to Dr. S. R. Haythorn for his kindness in preparing the photomicrographs used in this paper.

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DESCRIPTION OF PLATES

Plates 51–54

Fig. 1. Low-power field, showing the general structure of the lesion and the surrounding reaction.

Fig. 2. One angle of the lesion, showing the necrotic center filled with polynuclears and surrounded by a zone of granulation tissue containing giant cells. Capillaries are few and small.

Fig. 3. High-power field of the margin of the necrotic area, showing the margin of large mononuclear cells.

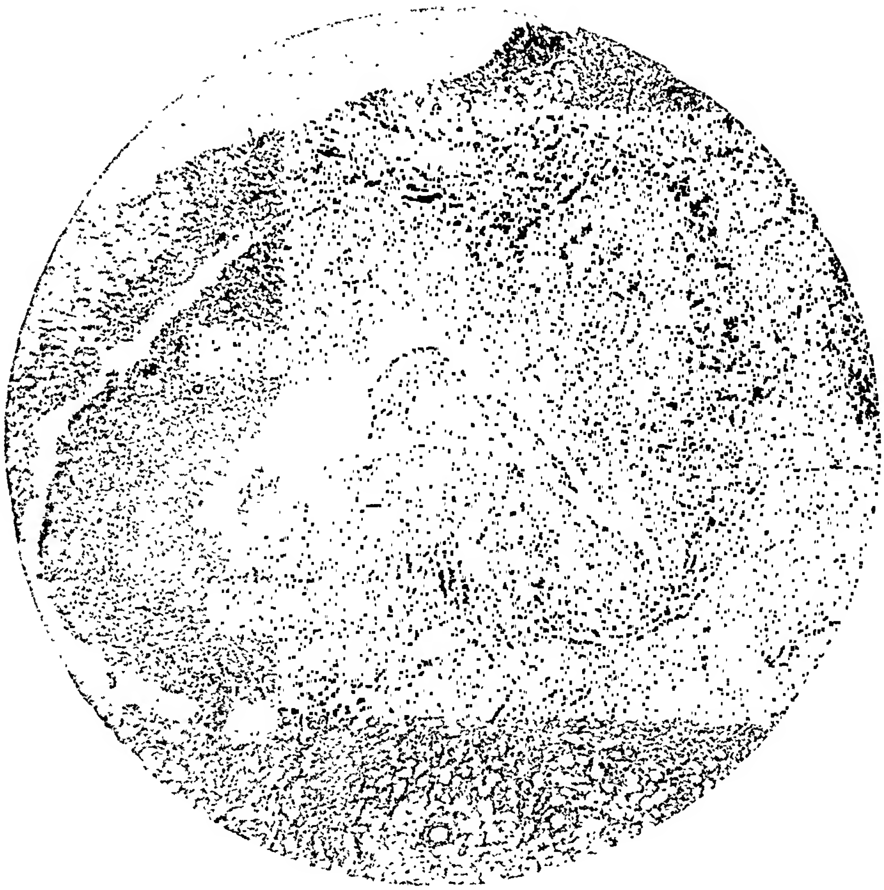
Fig. 4. Early secondary lesion in the fibroblastic wall, showing collections of mononuclear cells and giant cells.

Fig. 5. More advanced secondary lesion, showing beginning necrosis and a large giant cell at the margin.

Fig. 6. Extreme examples of proliferation of capillary endothelium in the wall of the lesion.

Fig. 7. Two mitoses in a capillary.

Fig. 8. Mononuclear collections and giant cell formation about injured adipose tissue cells in the areolar tissue involved in the reaction.



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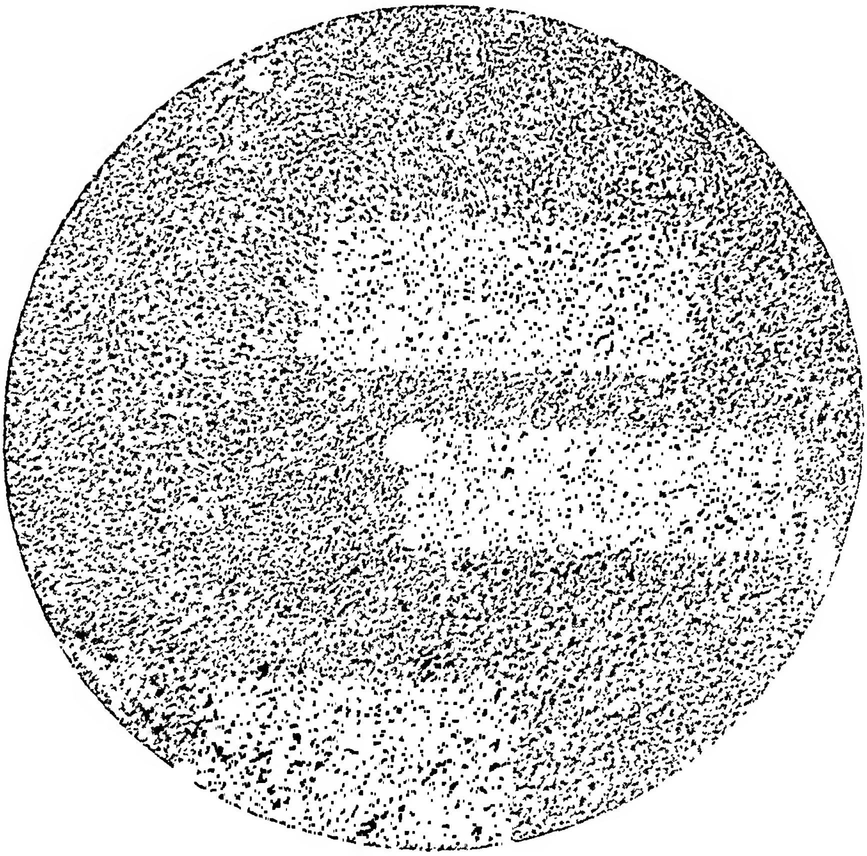

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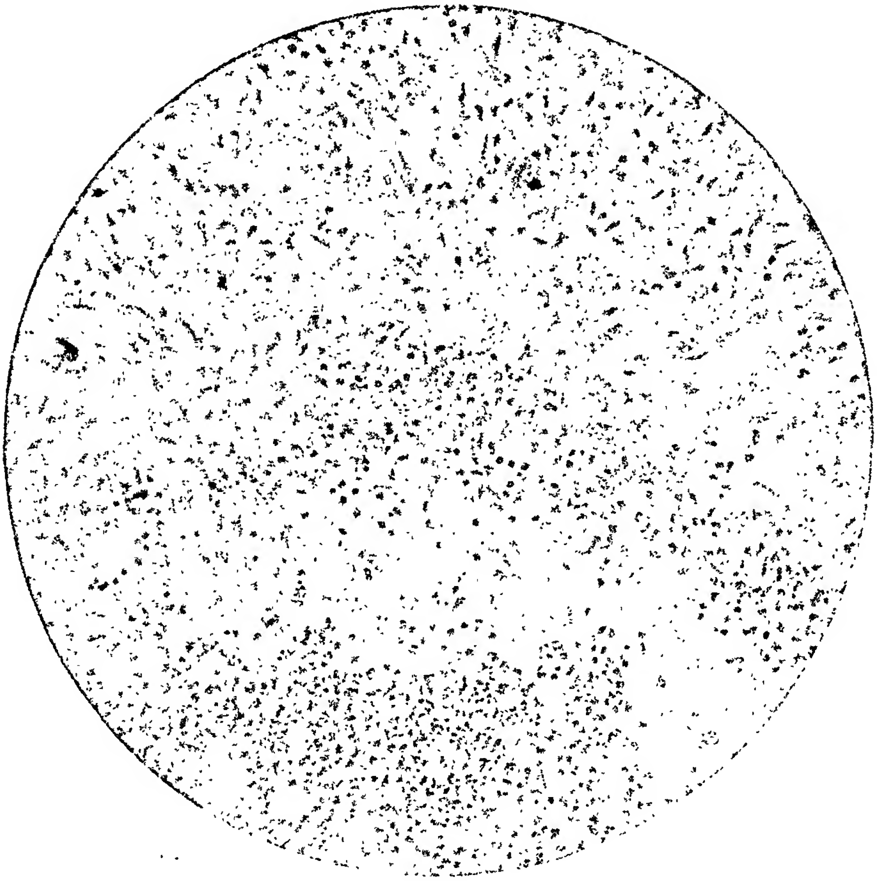

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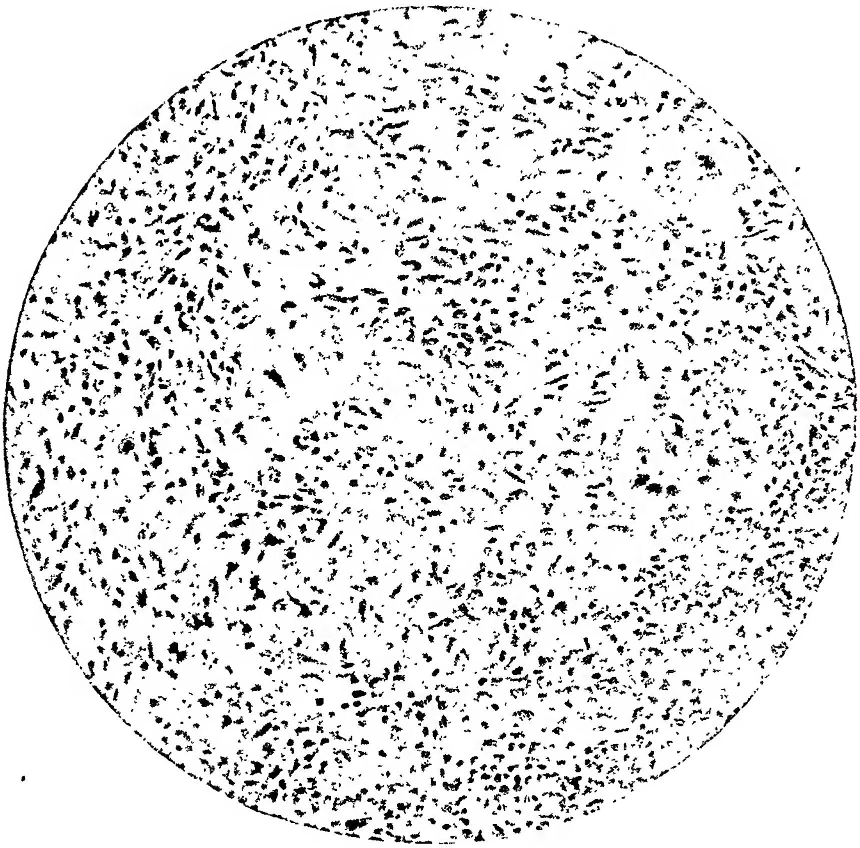

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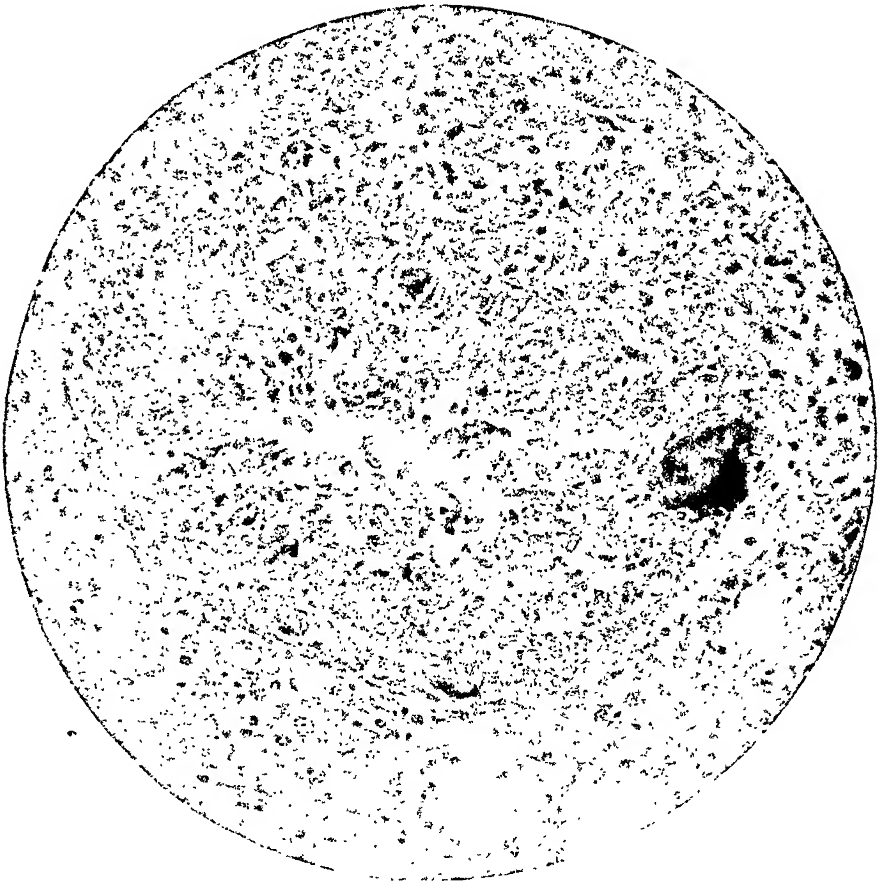

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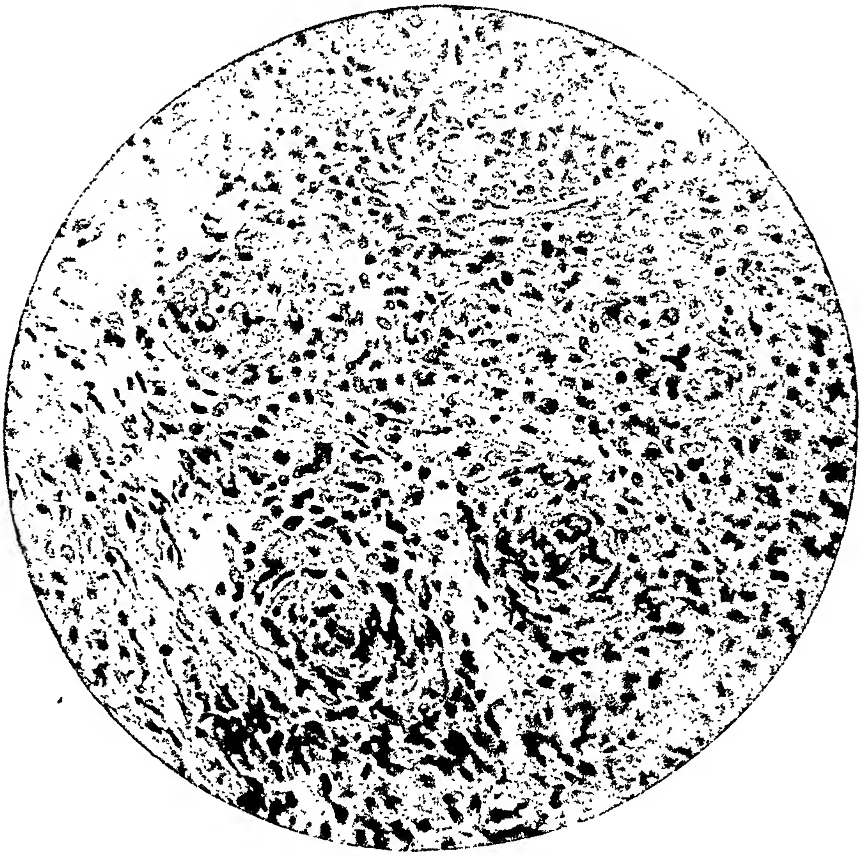

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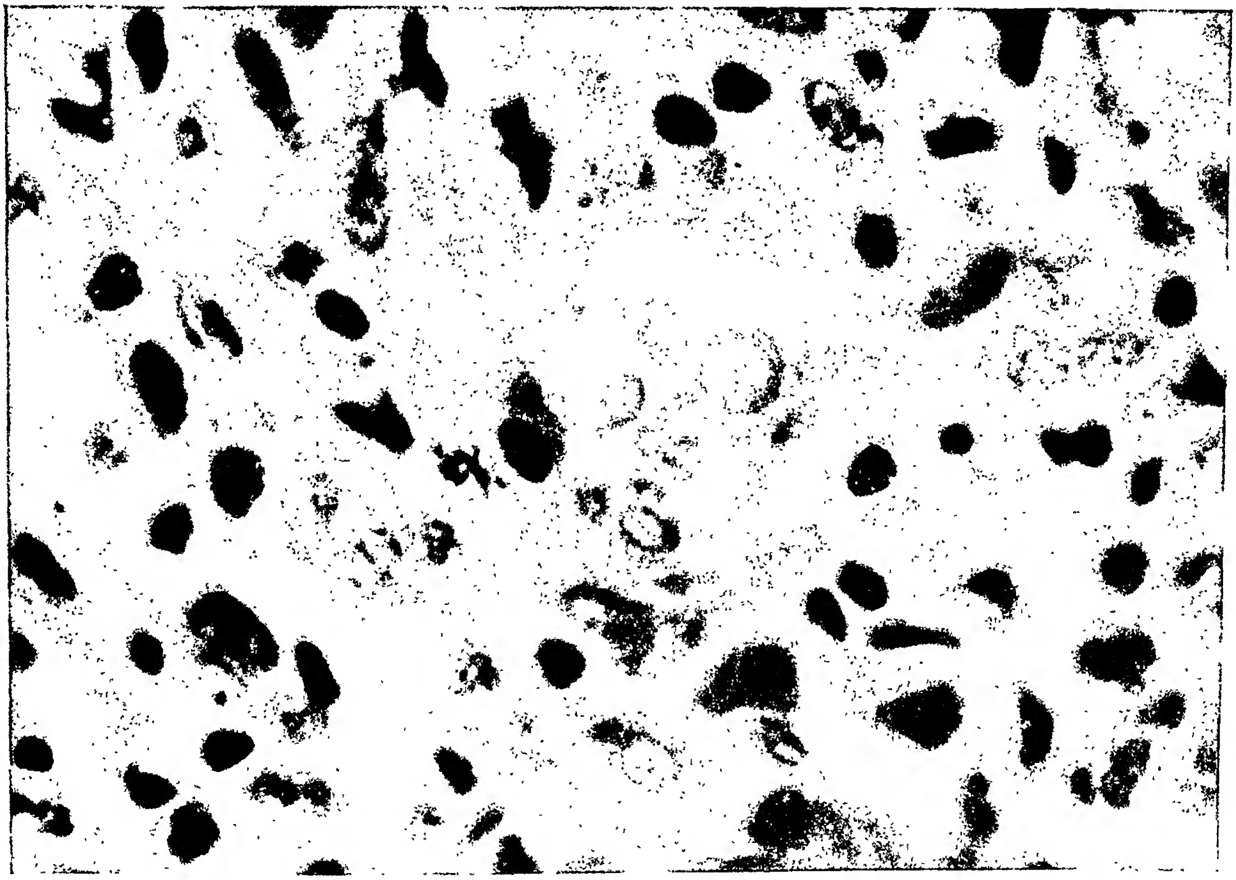

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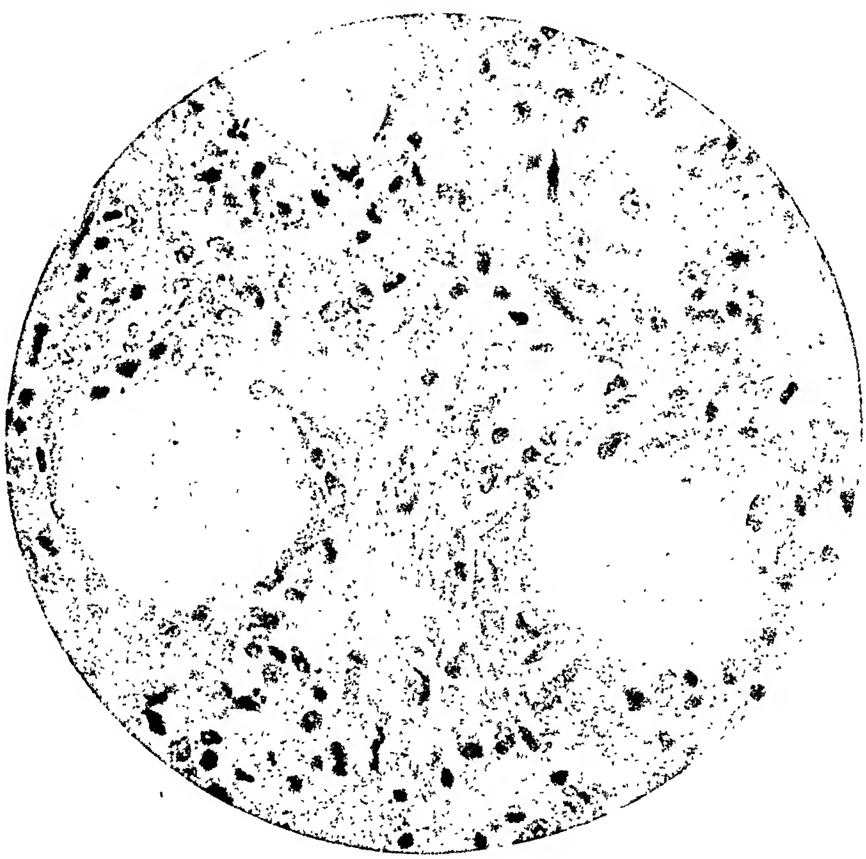

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A STUDY OF THE PROCESS OF CASEATION IN TUBERCULOSIS *

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The term caseation is applied to the gross characteristics of the necrotic material commonly seen in tuberculous infection. This necrotic tissue can be easily removed from the surrounding living tissue, leaving a cavity. It has no texture and varies in consistence from thick pus to a crumbly material not unlike cottage cheese. In these respects it differs from the necrotic tissue seen in a bland infarct or in a gumma, which cannot be readily removed from the surrounding viable tissue and which has a distinct rubber-like consistence. The necrotic material in an abscess differs from all of the above in that it is usually more liquid in character. Since these lesions are all, at times, seen in the same tissue and are either of an anemic or of an inflammatory nature, why should the necrotic tissue in tuberculosis differ from that seen in other conditions?

During the past four years an extensive study of the process of caseation has been undertaken. A large series of cases of both natural and experimental infection have been examined. Tuberculous lesions in the lung, spleen, liver, intestine, kidney, fallopian tubes and lymph nodes have been carefully studied by the serial section method. At least 200,000 sections have been carefully examined during this investigation and a large number of these sections have been examined for the presence of tubercle bacilli. Tissues from human cases obtained at necropsy and at surgical operations, from guinea-pigs inoculated with bovine and human tubercle bacilli and from fowl naturally infected with the avian strain of the tubercle bacillus have formed the basis of this study.

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Early in the study of the human tissues it became apparent that, if the natural sequence of events in the process of caseation was to be rightly understood, it would be an essential requirement to obtain tissues at known periods after infection. To obtain such tissues a series of guinea-pigs were inoculated on the same date with an equal dosage, approximately 20,000, of human tubercle bacillus, strain H37. The inoculation was made subcutaneously in the left groin. The skin over the area to be inoculated was carefully sterilized with iodine before inoculation and care was taken to avoid any contaminating infection. In none of the animals was there any evidence of acute inflammation on gross inspection at the site of inoculation at any time. Beginning with the tenth day after inoculation, animals were sacrificed every fifth day. The series gave tissues 10, 15, 20, 25, 30 and 35 days after the inoculation. Previous studies had shown that the spleen and the inguinal, iliac and par-aortic lymph nodes were the most uniformly infected following inoculation in the groin. These tissues were carefully studied for the presence of tubercle bacilli and the type of the inflammatory reaction.

Caseation in the Guinea-Pig

Since it is possible in the guinea-pig to follow the tuberculous process, step by step, from the earliest inflammatory reaction to the stage of typical caseation, a brief account of the process as it occurs in this host will be given first. This will be followed by a description, with an attempt at correlation, of the pathologic picture as seen in natural infection in the fowl and in man.

It is now common knowledge, dating back to the work of Yersin [1] and of Borrel,[2] that when tubercle bacilli are injected intravenously for the first time in the rabbit, the first inflammatory reaction occurs within the capillaries in the various tissues and organs where the bacilli lodge. This reaction is microscopic in proportion and consists of a varying proportion of polymorphonuclear and mononuclear leucocytes. Both types of cell phagocytize the bacilli. This first polymorphonuclear leucocytic reaction is never great and is of a transitory nature, hence abscess formation does not follow. The polymorphonuclear leucocytes which have been attracted die within a few days. From this stage on the mononuclear leucocytes continue to accumulate and the tubercle is formed.

This same reaction occurs at the site of the primary inoculation in the guinea-pig. This reaction, if the size of the dose be small, is never of sufficient extent to attract attention on macroscopic examination of the site. If the dosage be large the reaction may be sufficient in amount to cause slight ulceration of the overlying tissues. The ulceration is due, apparently, to the accumulation of polymorphonuclear leucocytes with resultant small abscess formation.

The distribution of the tubercle bacilli through the body from the primary locus has been so carefully and thoroughly studied by various authors that this phase of the subject will not be entered into. Suffice it to state that the bacilli are phagocytized by both the polymorphonuclear and mononuclear leucocytes which, by their wandering proclivities, first gain access to the lymphatics and later to the blood stream.

It is in these secondary foci of infection, where the bacilli are at first few in number, that the entire and probably characteristic reaction to tubercle bacillus infection can be found. Here it is possible to follow the development of the lesion from its beginning, step by step, to caseation and to healing, if the infection is overcome. This process can be followed in the various organs of the guinea-pig. The pathologic process as it develops in the spleen is here given. The spleen is chosen for three main reasons. In the first place it is of such size that serial section of the entire organ does not entail too great technical labor. Secondly, its infection with the tubercle bacillus is entirely hematogenous and, therefore, as remote as that of any organ from the original site of inoculation. And in the third place, it has no connection with the outside world, as do the lung and kidney, and consequently would be the least liable to be involved with infectious agents other than the one inoculated.

A comparative study of tuberculosis of the spleen in the human and the guinea-pig will be reported at a later date. Here the only phase taken up will be the pathologic process as it concerns caseation.

At ten days the spleen shows no tubercle formation. By careful and persistent search one may find a single bacillus or a group of two or three bacilli about midway between the splenic artery and the periphery of the Malpighian corpuscle. These bacilli are usually within mononuclear leucocytes, although some of them are, apparently, free in the tissue. The small groups of bacilli are the ones most often found free.

From this point on one finds the greatest development of the tuberculous process in the Malpighian corpuscles, though as time elapses one also finds an increasing number of small lesions developing within the histologic unit of the spleen pulp as described by Mall.[3] There is a gradual increase in the number of tubercle bacilli up to the twenty-five day specimen. This increase is due in all probability to two factors, namely, the multiplication of the bacilli in the organ and the continued accumulation of the bacilli filtered out of the blood stream. The latter factor would appear to be the predominant one, for one finds the bacilli scattered singly or in groups of two or three, largely within mononuclear leucocytes, throughout the developing lesion. One finds the bacilli distributed in somewhat of a collar arrangement in the outer portion of the splenic corpuscle and in the histologic unit.

The cellular reaction in the fifteen and twenty day specimens consists almost entirely of mononuclear leucocytes. In many areas nearly the entire Malpighian corpuscle or the histologic unit is filled and greatly enlarged by the accumulation of these cells. This is the mononuclear or "epithelioid" tubercle as it develops in the guinea-pig. Lymphocytes are very rare in the tubercle of the histologic unit but are numerous in the area outside of the tubercle in the Malpighian corpuscle. Polymorphonuclear leucocytes are very uncommon in the tubercle but may be found in small numbers in the tissue surrounding it. There is no evidence of caseation. One does find, however, mononuclear leucocytes with vacuolated cytoplasm and very faintly staining nuclei. Mitotic figures are commonly seen in the splenic pulp and less frequently are found in the tubercle itself. There is abundant evidence that the mononuclear leucocytes migrate into the tubercle, for one very commonly finds these cells with their nuclei stretched out in a long sinuous process.

Beginning, at times in the twenty day but more marked in the twenty-five day specimen, and continuing in the thirty and thirty-five day specimens, one sees a gradual and increasing accumulation of polymorphonuclear leucocytes in the areas where caseation later becomes apparent. These areas are often near the center of the tubercle. They may, however, be eccentrically placed. They vary in size from a small focus to an area which eventually becomes as large as many tubercles. The immigration and the multiplication

of the mononuclear leucocytes by mitosis do not entirely cease but the accumulation of the polymorphonuclear leucocytes far overshadows the mononuclear infiltration, so that in many instances the inflammatory reaction resembles abscess much more than tubercle formation. Bacilli are present, more numerous in some instances and fewer in others, when compared to the fifteen and twenty day specimens. But in none of the areas where the inflammatory reaction is most intense does the typical picture of caseous material appear at this stage. Many of the smaller foci, especially in the histologic units, do show the typical picture of caseation and if the dead cells are not too closely packed together one can make out with a fair degree of certainty the outlines of the nuclei of the polymorphonuclear leucocytes. These areas also contain a varying amount of nuclear "dust." In the larger areas one sees the beginning of caseation at the periphery and in numerous instances cells in the process of disintegration. An interesting finding in these areas is the presence of mononuclear leucocytes which have ingested from one to half a dozen polymorphonuclear leucocytes. Tubercle bacilli are quite commonly found within the polymorphonuclear leucocytes which have been engulfed. Lymphocytes are rare.

In lesions of six, seven or eight weeks' duration, the picture of caseation is present in many of the larger lesions. The necrosis appears to begin at the periphery of the most intense inflammatory reaction and gradually to proceed toward the center. There is more or less solution of continuity between the viable tissue at the periphery and the necrosing inflammatory exudate within, so that a modified abscess formation is the end result.

In the inflammatory process as described, giant cells are not found. They do not appear to be an essential part of the early inflammatory reaction or of tubercle formation, at least as it is produced in the guinea-pig.

As these areas of caseation in the spleen cannot be discharged to the outside without rupture of the splenic capsule, the cellular reaction subsequent to caseation can be followed. Polymorphonuclear leucocytes are not further attracted after caseation has been produced. Mononuclear leucocytes continue to accumulate and they gradually invade the caseous mass. Lymphocytes also are attracted in increasing numbers and likewise invade the caseous material. At

this stage of the reaction giant cell formation begins to take place. The significance of this phase of the reaction will be discussed in a separate paper on giant cell formation in tuberculosis.

In many of these areas of caseation, stainable bacilli are very few or absent. In other areas one finds colonies of stained bacilli. This presents a picture quite different from the even distribution of the bacilli, individually or in small groups throughout the lesions, in the earlier stages of the tuberculous process.

Quite often the inflammatory reaction is so extensive in the larger lesions that infarction of splenic tissue not primarily involved in the tuberculous process is produced. The size of the infarction varies with the size of the artery involved, but at times involves as much as half of the organ. This point is of considerable importance in the consideration of the essential tuberculous lesion. One has, however, little difficulty in distinguishing between the infarcted non-tuberculous tissue and the areas of tuberculous involvement. In the infarcted tissue one can distinguish easily between the areas of tuberculous inflammation and the non-tuberculous splenic areas which show the outlines of the sinuses and trabeculae of the dead splenic tissue. In fact, if the infarct be large, one finds several areas of tuberculous involvement within the infarcted area. These areas can be identified with certainty by the finding of tubercle bacilli, whereas the uninvolved infarcted tissue shows no bacilli.

Caseation in Avian Tuberculosis

Since one cannot determine the date of infection in cases of natral infection, it was impossible in the fowl to follow the development of the inflammatory reaction to the tubercle bacillus in time sequence as it was done in the guinea-pig. However, it seems logical that if stages of reaction corresponding to those observed in the guinea-pig can be found, the identity of the development of the inflammatory reaction to tubercle bacillus infection in the guinea-pig and in the fowl would be established. In the various fowl tissues studied it was possible to demonstrate numerous examples of typical mononuclear, or "epithelioid," tubercles without caseation, giant cell formation or polymorphonuclear leucocytic invasion. It was also possible to find numerous examples of tubercles without caseation but with polymorphonuclear leucocytic infiltration which

varied from a few to a large number of these cells in the areas where caseation occurred; and examples of different stages of caseation were numerous. In brief, all the stages leading up to the typical picture of caseation present in the guinea-pig were easily demonstrated in the fowl tissues naturally infected with the avian strain of the tubercle bacillus.

While it is not pertinent to go into a full discussion of the pathologic picture as seen in avian tuberculosis, there are a few facts worthy of comment. In the first place tubercle bacilli are far more numerous in the lesions than have been found in either guinea-pig or human lesions. In the caseous material large masses of bacilli in colonies are very commonly found; and in these same areas one finds large numbers of "phantoms" of unstained bacillary forms which correspond in size, shape and distribution to the stained bacilli.

In the fowl the leucocyte which corresponds to the neutrophilic polymorphonuclear leucocyte in man has an abundance of eosinophilic granules. This fact makes for easy recognition of these cells and when they are massed together in the midst of a tubercle they take an intense eosin stain which contrasts markedly to the blue-staining of the mononuclear leucocytes clustered around the periphery of the mass. The caseous material early takes a much more intense eosin stain than it does in man or guinea-pig. This fact is strongly suggestive of the important part played by the polymorphonuclear leucocyte in the production of caseous material in the fowl.

As in the guinea-pig, the polymorphonuclear leucocyte does not appear to be attracted by the caseous mass after it is once produced. In fact it is quite common to find large areas of polymorphonuclear infiltration directly adjacent to an old area of caseation with no evidence of the polymorphonuclear leucocytes attempting to invade the old caseous mass.

The mononuclear leucocyte appears to play the same rôle in the inflammatory reaction in naturally acquired avian tuberculosis as it does in the guinea-pig experimentally infected with the human or bovine tubercle bacillus. In freshly fixed tissues, one finds abundant evidence of its motility, as shown by greatly elongated nuclei. In the mononuclear tubercle, it is found to have tubercle bacilli within it. Mitotic figures in these cells are fairly easy to find in and about the tubercles.

The lymphocyte is very commonly found in the tissues surrounding the tubercle and the caseous material. It does not appear to take any active part in the formation of the tubercle or in the process of caseation.

Giant cells are usually far more numerous than in the guinea-pig tissues or in the human material I have studied.

Caseation in Human Tuberculosis

As in the case of the fowl, the human cases were those of natural infection and no date of the initial infection could be established. It would seem that the same logic which applied to the correlation of the avian and guinea-pig tuberculous reactions would obtain in man. Stages from the mononuclear tubercle without giant cell formation, polymorphonuclear infiltration or caseation to the typical picture of caseation were found, not once but many times, in the spleen, lymph node, kidney, liver, fallopian tube and lung. So that it would appear that there is close similarity between the inflammatory reaction and the appearance of caseation in guinea-pig, fowl and man.

The caseous material in man is composed in large part of dead polymorphonuclear and mononuclear leucocytes, but it appears that the bulk of the necrotic material is made up of polymorphonuclear leucocytes. In many of the areas the inflammatory cells are so closely packed together that it is impossible to make out the outline of individual cells. The intense hematoxylin stain which these areas take during the process of cell disintegration is another fact which is evidence of the marked cell accumulation during the most active inflammatory phase. The large amount of nuclear "dust" seen in the later phase of the process can readily be explained in the same way. In the later phases of cell disintegration it is impossible to determine the types of cell which compose the compact caseous mass. But if one may judge from the earlier steps in the process the lymphocyte plays little or no part.

The cellular reaction which occurs after the process of caseation is complete, that is, when the caseous material no longer shows nuclear "dust," differs in some respects from that which takes place before caseation is produced. Around these areas one finds many mononuclear leucocytes and a varying number of lymphocytes. Both of these cell types wander into the caseous material and can

be found all the way through it if the area is not too large. Polymorphonuclear leucocytes are not present in the reaction at this time and appear to take no part in the removal or organization of the caseous material. At this stage giant cell formation begins.

Among the human tissues examined were several cases of the hyperplastic type of tuberculosis. The majority of these were in lymph nodes removed surgically. A study of the inflammatory reaction in these cases showed a variation from the process described above in several respects. Mononuclear or "epithelioid" tubercles are much more common than in the caseating type of tuberculosis. Lymphocytes are more plentiful in the tubercles, often having accumulated in considerable numbers in the small areas of necrosis of the mononuclear leucocytes and in the rare areas of typical caseation. Polymorphonuclear leucocytes are extremely rare, except where typical caseation is being produced. Areas of caseation are rare and are small when present. Fibrosis is very common, many of the tubercles showing little but hyaline collagen fibers. Giant cells are present but are not numerous. Tubercle bacilli are scarce, the finding of a single tubercle bacillus being a laborious task.

Discussion

If the steps in the process of caseation in the guinea-pig, fowl and man as described above are a correct interpretation of the development of the tuberculous process, then it would appear that the process is the same regardless of the type of tubercle bacillus causing the infection or of the host infected. When caseation appears it is an indication of the virulence of the infection, of the lack of resistance of the host, of the dosage or of all three. When hyperplastic tuberculosis without caseation develops it is an indication of low virulence on the part of the bacillus, good resistance on the part of the host, small dosage or a combination of these factors. When caseation develops in a situation where it is possible for the necrotic material to be extruded from the body, as in the lung, kidney, skin or intestine, cavitation or ulceration ensues. If caseation occurs in a place, such as bone, where the caseous material cannot be extruded, a cold abscess will develop if the process is not successfully walled off. In case the caseous area is successfully walled off, it will either become calcified or be organized and a scar produced.

The term "mononuclear leucocyte" used throughout the description of the tuberculous process is, perhaps, not the best term to denote the cell. It is the ameboid phagocytic cell which has to do with tubercle and giant cell formation. It is apparently the same cell met with in typhoid fever, blastomycosis and other diseases and pathologic conditions where the function of phagocytosis of material that cannot be successfully handled by the polymorphonuclear leucocyte, is of vital importance. It is the cell of varied terminology. It has been designated as the epithelioid cell, the wandering mononuclear leucocyte or phagocyte, the macrophage of Metchnikoff, Evans and others, the endothelial leucocyte of Mallory, the polyblast of Maximow and the monocyte of Cunningham and Sabin. While its exact origin is perhaps still unsettled, its activities are fairly well understood. Its participation in the tuberculous process is generally accepted. A cell capable of ameboid motion, such as this cell possesses, could migrate to the site of the tubercle bacillus from the blood stream or from the surrounding tissues with equal ease. If one may judge by the presence of mitotic figures, it also multiplies after arrival at the site of infection. Whatever its name or its origin, it appears to be the greatest single factor in the destruction of the tubercle bacillus that the body possesses. It is my belief that the number of tubercle bacilli destroyed by cells of this type during tuberculous infection is but little realized.

It is the chief intent of this paper to direct attention to the rôle which the polymorphonuclear leucocyte seems to play in the process of caseation. In the mononuclear or "epithelioid" tubercle, the polymorphonuclear leucocyte is not seen until there is evidence of damage to the mononuclear leucocytes. This damage is shown by a slightly greater eosin staining of the cytoplasm, by loss of nuclear structure and oftentimes by vacuolization of the cell cytoplasm. This does not resemble caseous material. In these small areas one can often make out bacillary forms which do not stain with carbolfuchsin. This can best be demonstrated in fowl tissues where tubercle bacilli are numerous in the tubercle. Whether the polymorphonuclear leucocytes are attracted by the necrotic mononuclear leucocytes, by opsonins, by substances liberated through the death of the tubercle bacillus or by substances produced in the tissues by the multiplying bacilli is not understood.

The idea which one gains from the literature is that if the poly-

morphonuclear leucocyte is present in the inflammatory reaction to the tubercle bacillus it is there because of secondary infection. Mallory [4] states that these cells are present only when the tubercle bacilli are very numerous. He also states that caseation is due "chiefly or entirely to endothelial leucocytes plugging the lymph or blood vessels, thus cutting off nutrition" and that "the necrotic material consists largely of endothelial leucocytes." MacCallum [5] states that even in acute tuberculous inflammation "polymorphonuclear leucocytes seem to be very little attracted, their place being taken by lymphoid cells and large mononuclear wandering cells." Aschoff [6] does not mention the polymorphonuclear leucocyte in connection with caseation which he considers to be caused by the necrosis of the epithelioid cells which form the tubercle. Wells [7] quotes Schmoll as finding that caseous material, even when it breaks down and forms a cold abscess, shows much less evidence of autolysis than pus. Maximow [8] claims to have produced caseation in tissue culture where the polymorphonuclear leucocyte, because it is not regenerated in such cultures, could have but little to do with the process. He obtained in tissue culture necrotic "epithelioid" cells but it is doubtful whether this can rightly be compared to the process of caseation as it occurs in living tissues where all types of cell have free access to the site of infection. Smith [9] states that the polymorphonuclear leucocyte does not play any recognizable rôle in the tuberculous process. He also states that the appearance of the polymorphonuclear leucocyte in the early reaction to cultures inoculated intravenously is due in all probability to the suspending fluid and to disintegrating bacilli which are always present in the cultures of a few weeks' growth.

Kostenitsch and Wolkow [10] speak of a primary polymorphonuclear leucocytic infiltration which occurs a few hours up to three days after the primary inoculation of tuberculous material, and of a secondary polymorphonuclear leucocytic infiltration which occurs at the time of caseation. As tubercle formation can take place up to the stage of caseation without the polymorphonuclear leucocyte participating, it appears that the polymorphonuclear leucocyte does not in reality make a primary and secondary appearance. It is evident that there is a phase in the reaction to the tubercle bacillus where a substance is produced which definitely attracts this cell. They also state that the primary reaction is more intense than the

secondary reaction. I have found the polymorphonuclear leucocytic response much more intense after well established infection has been produced.

There is abundant evidence that the polymorphonuclear leucocyte is not attracted by caseous material after it is once formed. This can be easily demonstrated in both experimentally and naturally infected tissues. The active participation of the polymorphonuclear leucocyte in the steps leading up to caseation can be as readily demonstrated if the tissues are examined at the proper stage of the lesion. The most conclusive demonstration of this can be made in avian tuberculosis because of the easy differentiation with certainty between the polymorphonuclear leucocyte and the mononuclear elements. The eosinophilic granules in the polymorphonuclear leucocyte of the fowl allow of this sure differentiation. Although there are no eosinophilic granules in the guinea-pig or human polymorphonuclear leucocyte, these cells can be identified with certainty until they are so closely packed together in the inflammatory reaction that cell outlines can no longer be determined.

Miller [11] and others have conclusively shown the existence of reticulum which is continuous with the surrounding tissue, as a constant feature of the well formed "epithelioid" tubercle. As long as this reticulum is intact, even if death of the mononuclear leucocytes occurs, it hardly seems possible that cavitation could be brought about. During the process of caseation, however, this reticulum may be more or less destroyed and its continuity with the surrounding viable tissue broken. Because of this fact it can readily be understood why caseous material in some cases is discharged to the outside when opportunity occurs and cavitation or ulceration results. The persistence of this reticulum, even in caseous material, would also explain why in other cases cavitation or ulceration is not produced. It would seem quite probable that the destruction of this reticulum is brought about by the polymorphonuclear leucocyte with its proteolytic enzyme rather than by the mononuclear leucocyte which appears to be very closely associated with the production of the reticulum in the mononuclear tubercle.

A certain parallelism between the lipoid content of pus and of caseous material would indicate that they are probably composed largely of the same cellular elements. According to the chemical analysis of dried pus by Hoppe-Seyler,[12] the lipoid content was

21.78 per cent and 22.34 per cent in two specimens. Bossart [13] reported the lipoid content of pure caseous material as 20.75 per cent of the dried stuff. The cholesterol content varied from one-third to one-fourth of the lipoid content in the caseous material, while in pus it was approximately one-third of the lipoid content. In the dried pus there is 7.2 per cent of lecithin and in dried caseous material there is 3.83 per cent according to an analysis by Schmoll and F. Müller.[14] While the lipoid content is not identical in pus and caseous material, there is sufficient similarity to suggest that their composition is quite alike. As the ages of the pus and caseous material chemically examined without doubt were different, the caseous material being the older, it would not be surprising if the lipoid content had differed more than the analysis showed.

From the study of the inflammatory reaction early in the process of caseation in the guinea-pig, it is apparent that the polymorphonuclear leucocytes are injured before disintegration takes place. One can easily find mononuclear leucocytes which have phagocytized from one to half a dozen well formed polymorphonuclear leucocytes. Stewart, Long and Bradley [15] have recently reported this observation in tuberculin and reinfection reactions. This phenomenon appears in the areas of primary infection as well as in the reinfection and tuberculin reactions. This picture is quite comparable to the phagocytic action which the mononuclears display for lymphocytes and erythrocytes in typhoid fever. This early injury to the polymorphonuclear leucocyte might explain why autolysis and softening do not take place in the process of caseation as in an ordinary abscess. Several investigators have failed to find any evidence of autolytic ferments in caseous material. Jobling and Petersen [13] suggest that this inhibition of autolysis is due to the presence of unsaturated fatty acids derived from the tubercle bacillus. As the autolytic ferment of the polymorphonuclear leucocyte requires an alkaline medium to enable it to bring about liquefaction, it would seem plausible that an acid reaction might be the cause for the failure of the leucocyte to bring about liquefaction as it does in a pyogenic infection. It would seem that some such phenomenon rather than a marked difference in the cellular content of caseous material and pus would be a logical explanation for the difference between caseation and abscess formation.

Conclusions

Caseation, cavitation and ulceration in tuberculosis appear to be due to the active participation of the polymorphonuclear leucocyte in the inflammatory reaction to the tubercle bacillus.

The process of caseation appears to be the same in naturally acquired tuberculous infection in fowl and man and in laboratory animals experimentally infected.

The presence of the polymorphonuclear leucocyte in tuberculous inflammation is not an indication of secondary infection with pyogenic bacteria. It is an indication of the production by the growth or death of the tubercle bacillus or by the action of the tubercle bacillus upon the tissue in the tubercle, of a substance which strongly attracts the polymorphonuclear leucocyte.

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EXPLANATION OF PLATES

Plates 55–61

Fig. 1. Guinea-pig spleen. Mononuclear tubercle in histologic unit of the pulp. Twenty days after groin inoculation. The tubercle is composed of mononuclear leucocytes, probably of reticulo-endothelial origin. In such a tubercle lymphocytes and polymorphonuclear leucocytes are extremely rare. No caseation. x 350.

FIG. 2. Guinea-pig spleen. Twenty-five days after groin inoculation. This tubercle is in the same anatomic position as the one in Fig. 1. Note the considerable number of polymorphonuclear leucocytes in the central portion. Lymphocytes are rare. There is no evidence of caseation. x 350.

FIG. 3. Guinea-pig spleen. Thirty days after inoculation in the groin. The lesion is in a histologic unit of the pulp. The dark mass is composed very largely of polymorphonuclear leucocytes. Lymphocytes very rare. No evidence of caseation. x 350.

FIG. 4. Guinea-pig spleen. Thirty-five days after groin inoculation. The lesion is in the outer portion of a Malpighian corpuscle. The dark mass is made up largely of polymorphonuclear leucocytes. Caseation is beginning as is shown by the hazy appearance of the tissue at the periphery. Lymphocytes are present in small numbers in the tissue around the caseating area. x 350.

FIG. 5. Guinea-pig spleen. Thirty-five days after groin inoculation. The lesion is in a histologic unit of the pulp. There is considerable nuclear "dust" in the center with typical caseation in the periphery. Note the predominance of mononuclear leucocytes around the area in the periphery of the caseous material. Several of these cells show elongated nuclei, presumably in the process of migrating into the caseous area. Lymphocytes are more numerous than in the preceding figures. Polymorphonuclear leucocytes while present are few in number. x 350.

FIG. 6. Avian tuberculosis. Typical mononuclear tubercle in turkey liver. Natural infection. Acid-fast stain. Corresponds to Fig. 1. x 350.

FIG. 7. Avian tuberculosis. Natural infection. Spleen of chicken. The dark staining cells are polymorphonuclear leucocytes. There is beginning caseation. x 350.

FIG. 8. Avian tuberculosis. Natural infection. Spleen of chicken. More marked accumulation of polymorphonuclear leucocytes than Fig. 7. This corresponds to Fig. 3. There was no evidence of caseation in the entire lesion which was serially sectioned. x 350.

FIG. 9. Avian tuberculosis. Natural infection. Liver of chicken. The dark area in the center is a compact mass of polymorphonuclear leucocytes. There is beginning caseation at the periphery. Note the vacuolated mononuclear leucocytes surrounding the area of polymorphonuclear leucocytes. This lesion corresponds to Fig. 4. x 350.

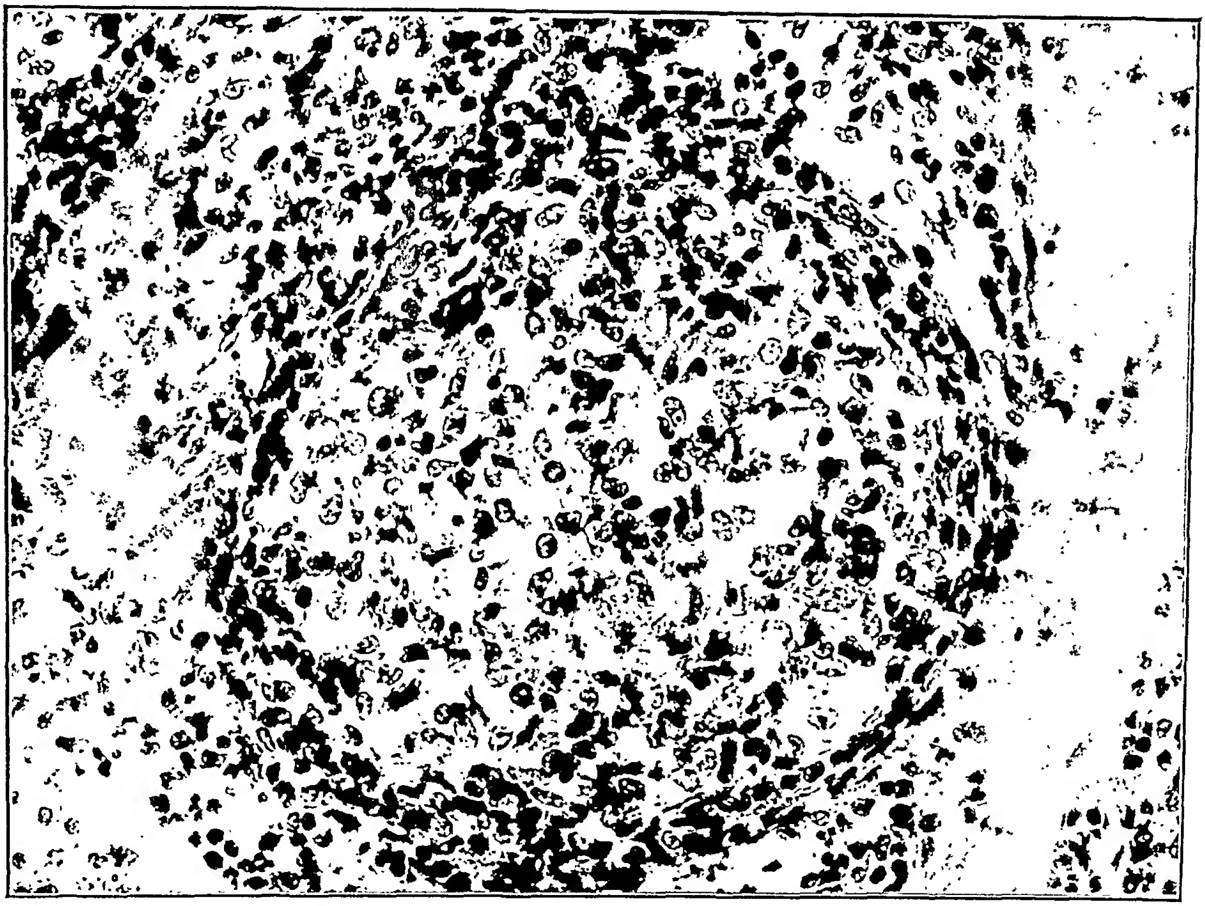
FIG. 10. Avian tuberculosis. Natural infection. Turkey liver. The lesion to the left shows old caseation and corresponds to Fig. 5. The lesion to the right shows a much greater accumulation of polymorphonuclear leucocytes but is about the same stage as Fig. 9. The polymorphonuclear leucocytes do not appear to be attracted by the older caseous material. Note the sharp line of demarcation between the two lesions. x 100.

FIG. 11. Human tuberculosis. Human spleen. Mononuclear tubercle. There is no caseation. Note the zone of polymorphonuclear leucocytes in the periphery of the tubercle. x 200.

FIG. 12. Human tuberculosis. Fallopian tube. Compare with Fig. 3. Note the elongated polymorphonuclear and mononuclear leucocytes, presumably migrating into the tubercle. There is no evidence of caseation. Lymphocytes are very rare. x 350.

FIG. 13. Human tuberculosis. Lymph node. The edge of a large lesion corresponding in age to Fig. 4. There is a lymphocytic zone above, a mononuclear zone in the middle and polymorphonuclear leucocytes below. Compare the nuclear shapes below with the nuclei of the polymorphonuclear leucocytes in Fig. 12. X 350.

FIG. 14. Human tuberculosis. Kidney. A small area of caseation. Compare with Fig. 5. Note the mononuclear leucocytic zone around the area of caseation, the nuclear "dust" in the area of caseation and the rarity of polymorphonuclear leucocytes at this stage. X 350.



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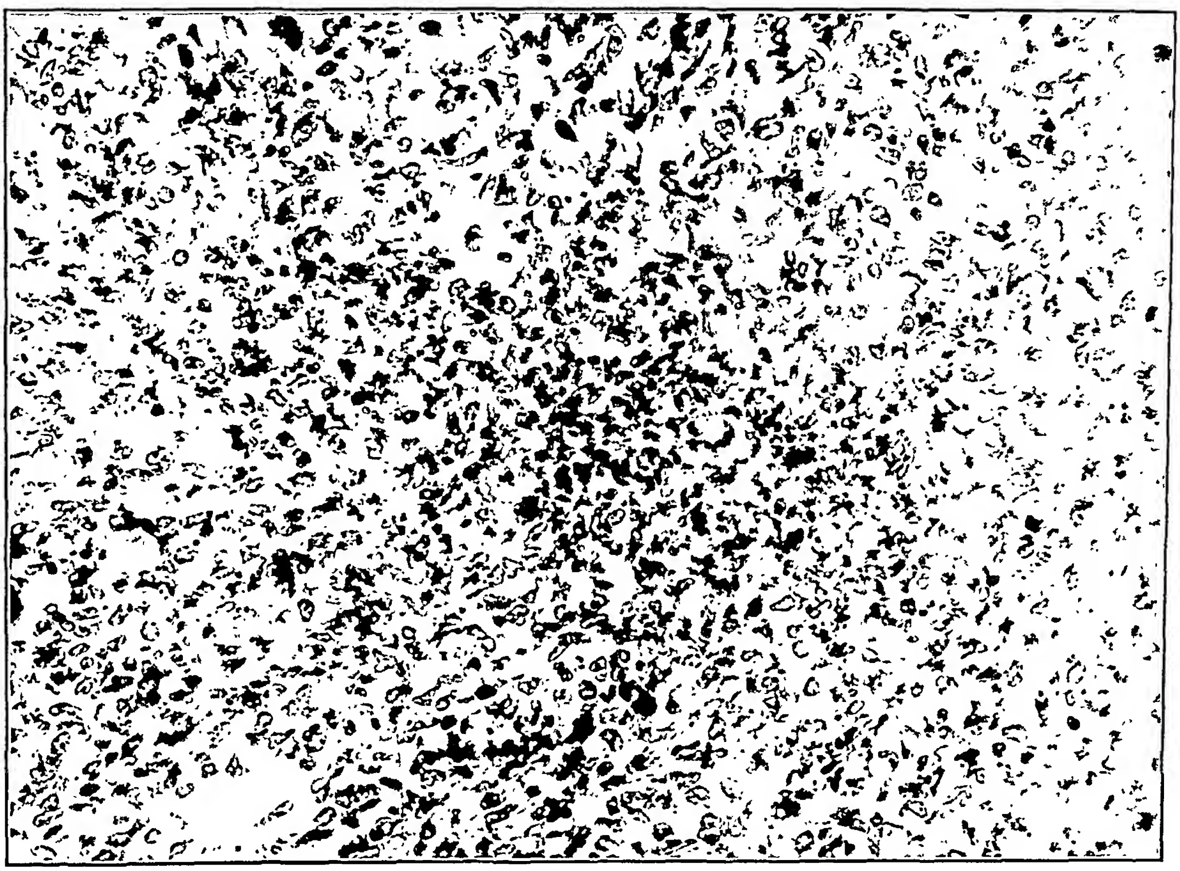

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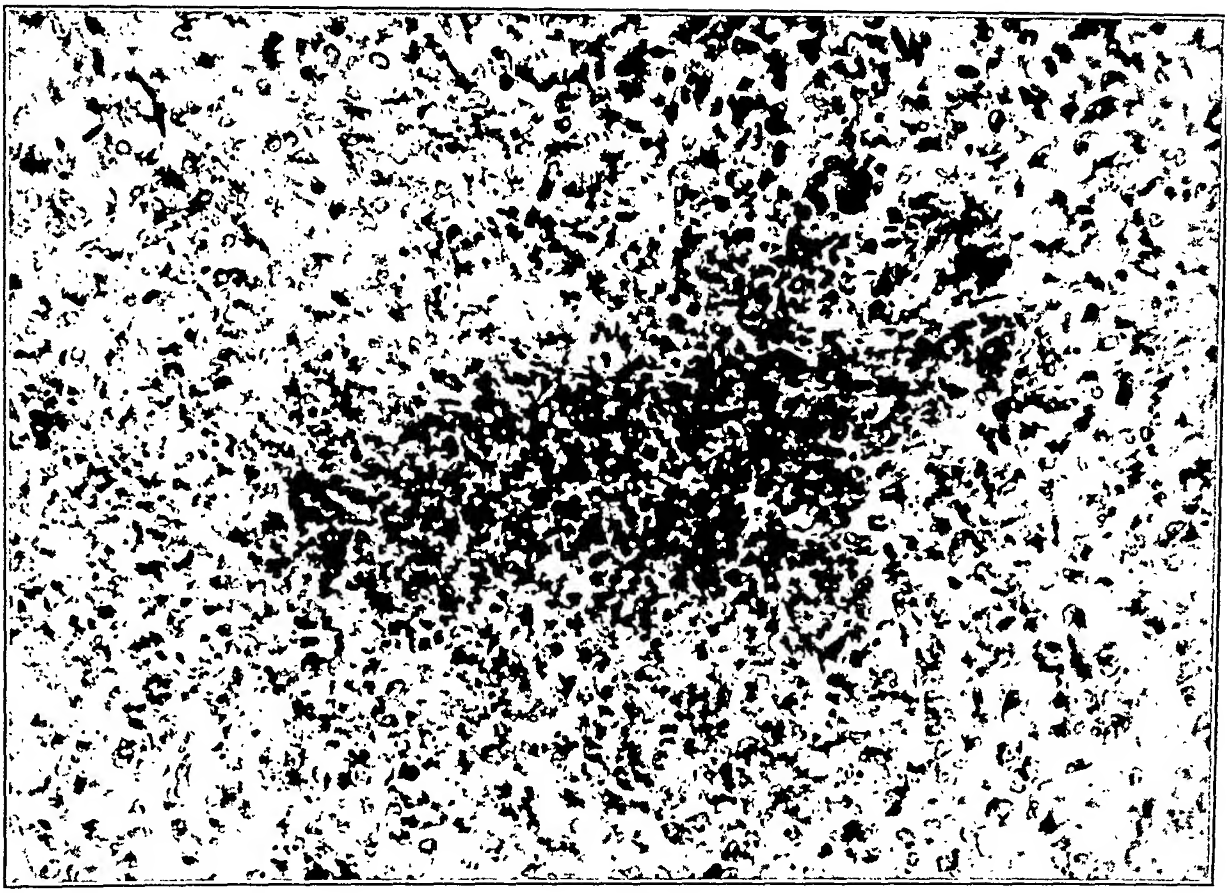

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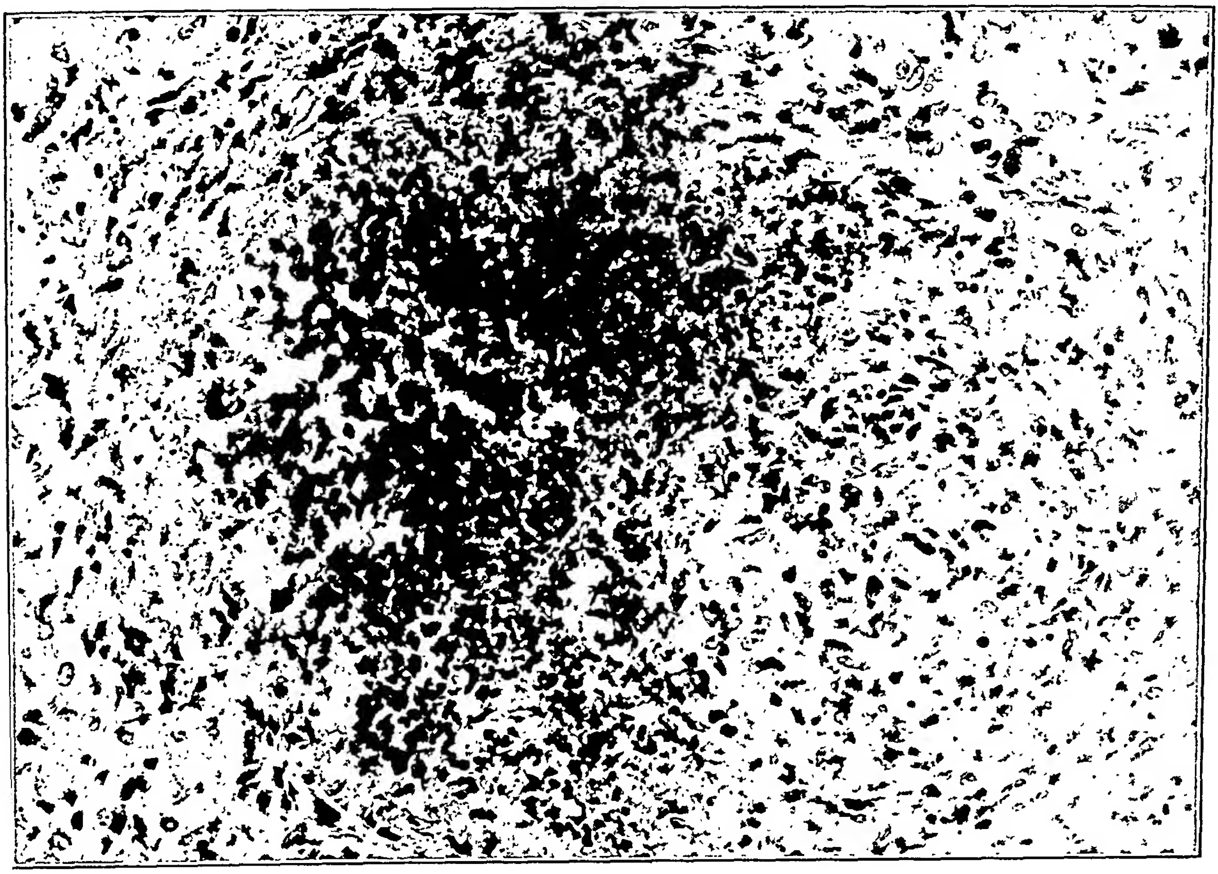

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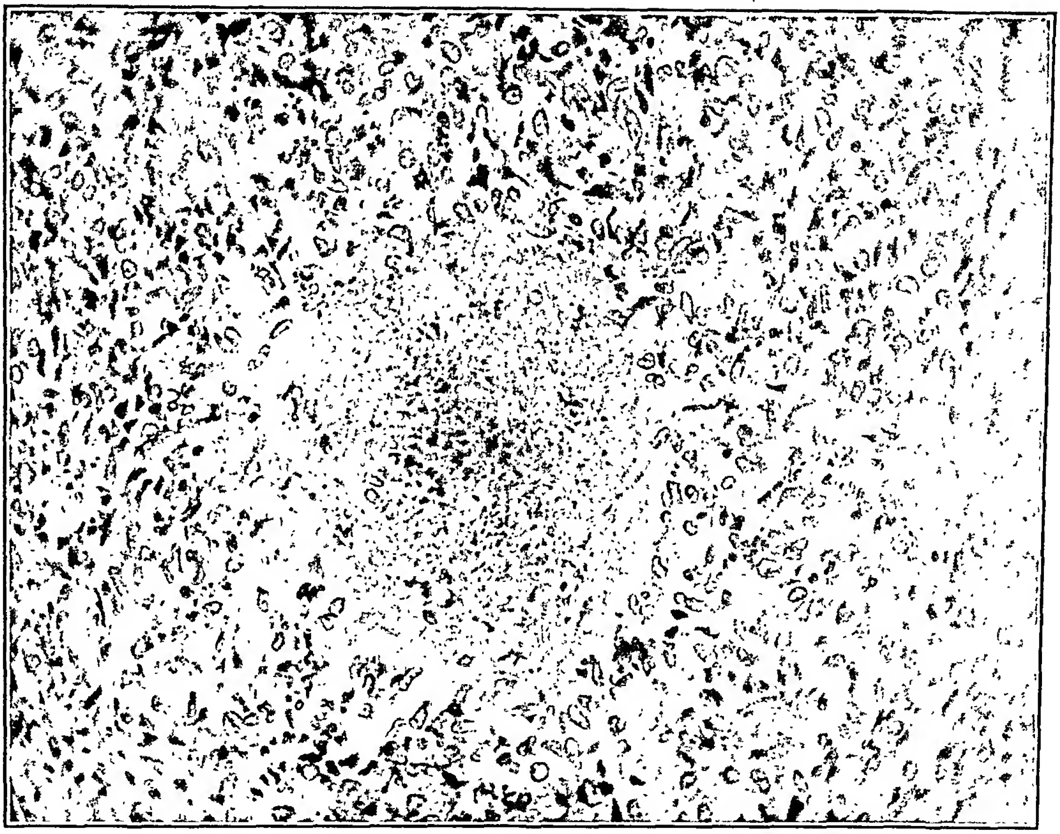
Caseation in Tuberculosis



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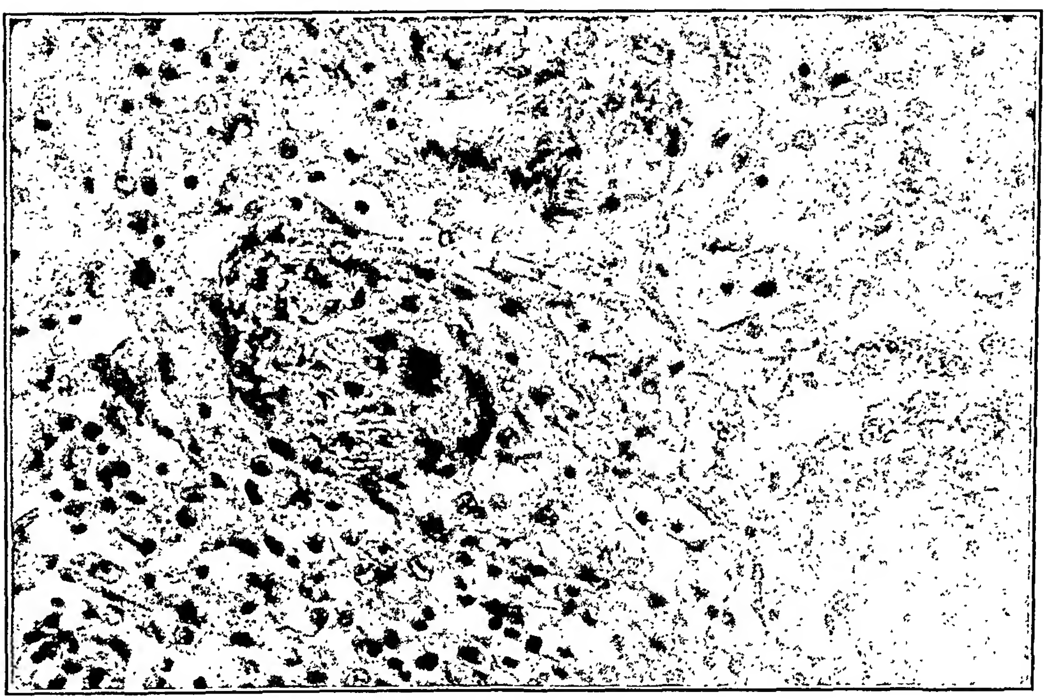

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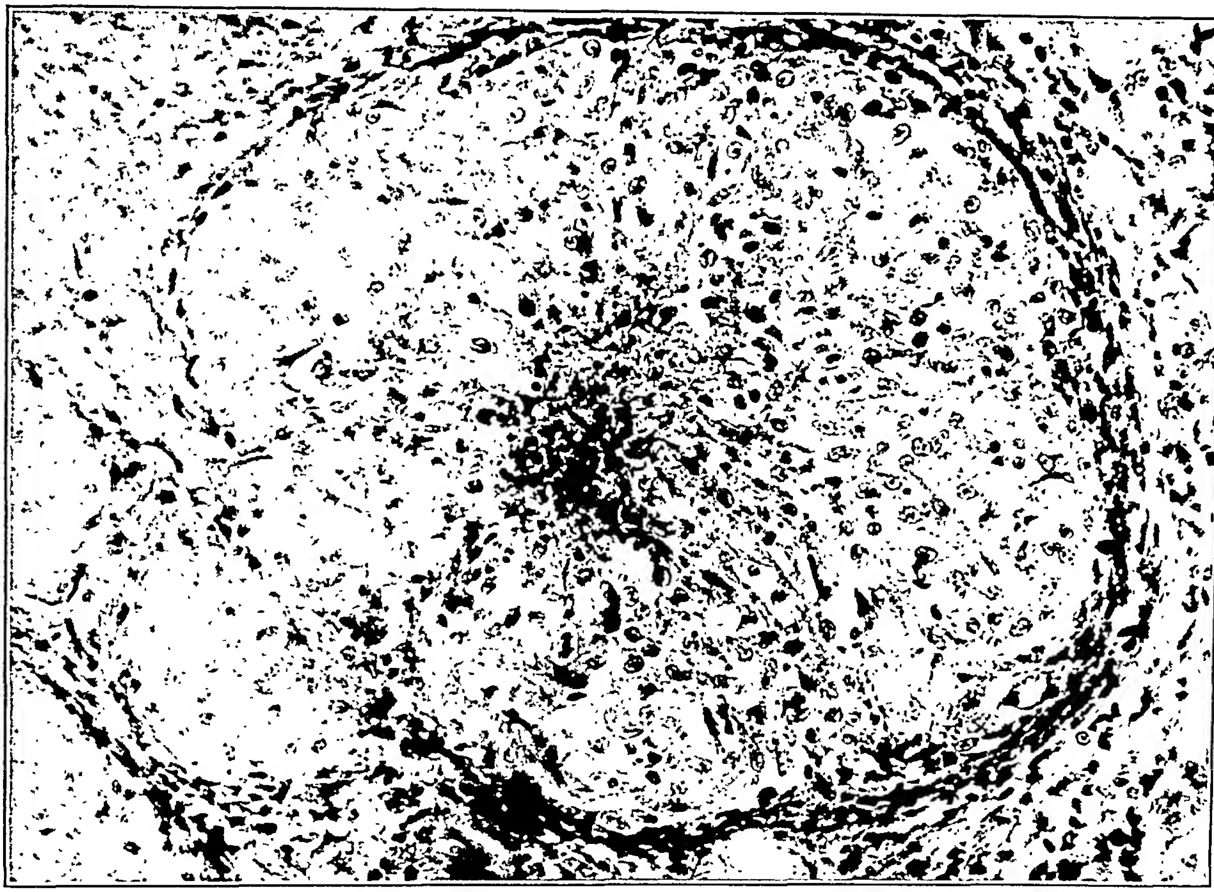

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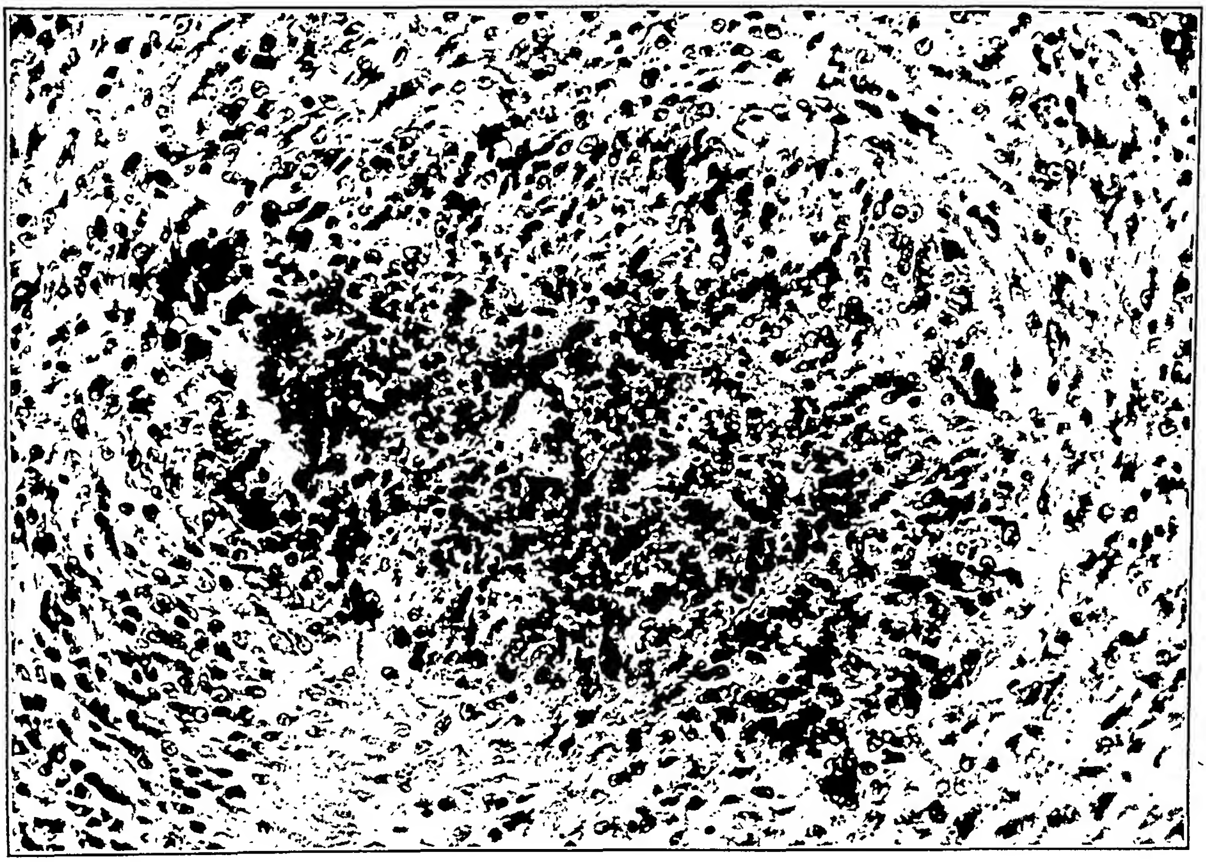

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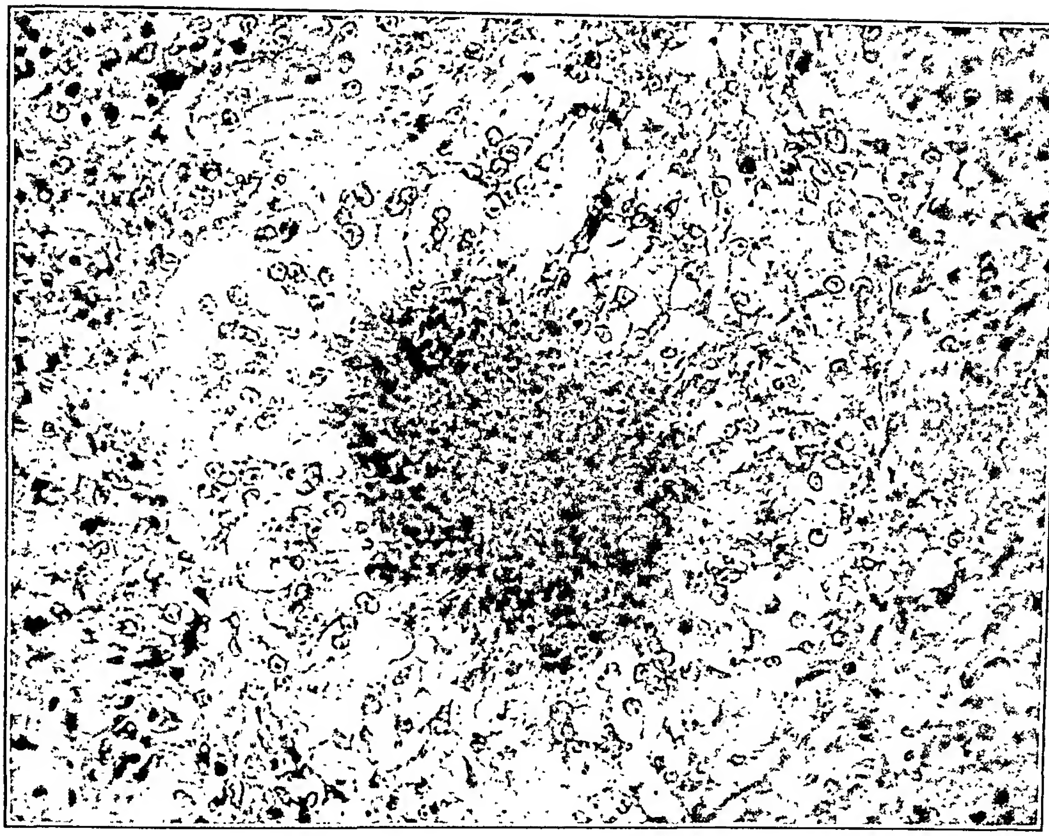

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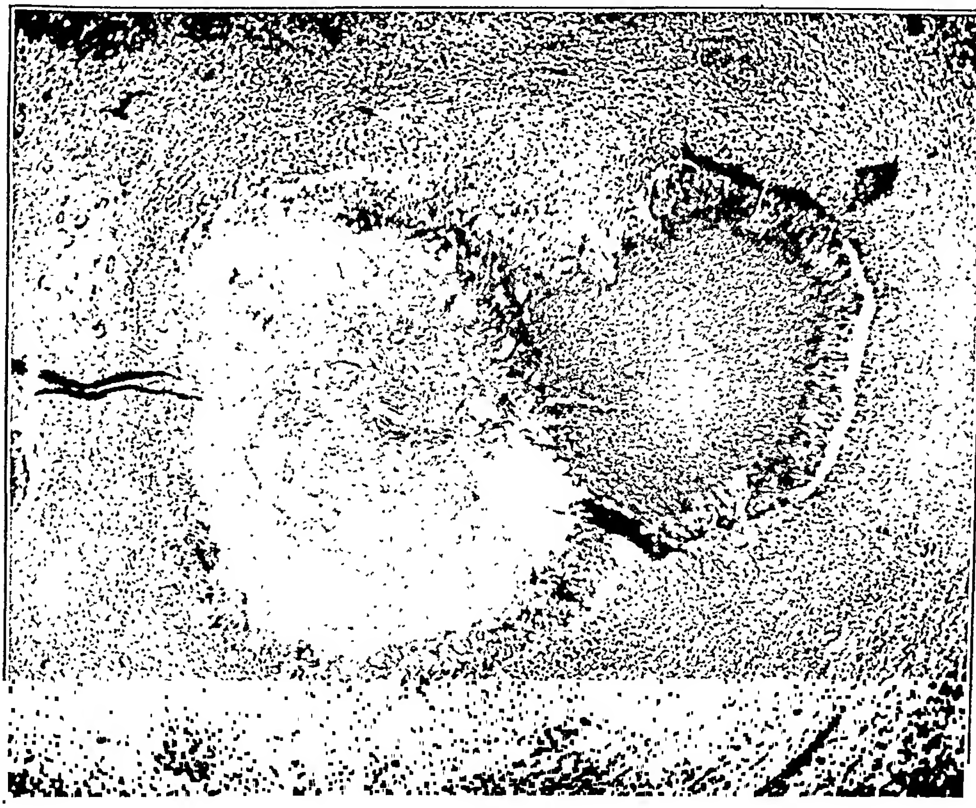

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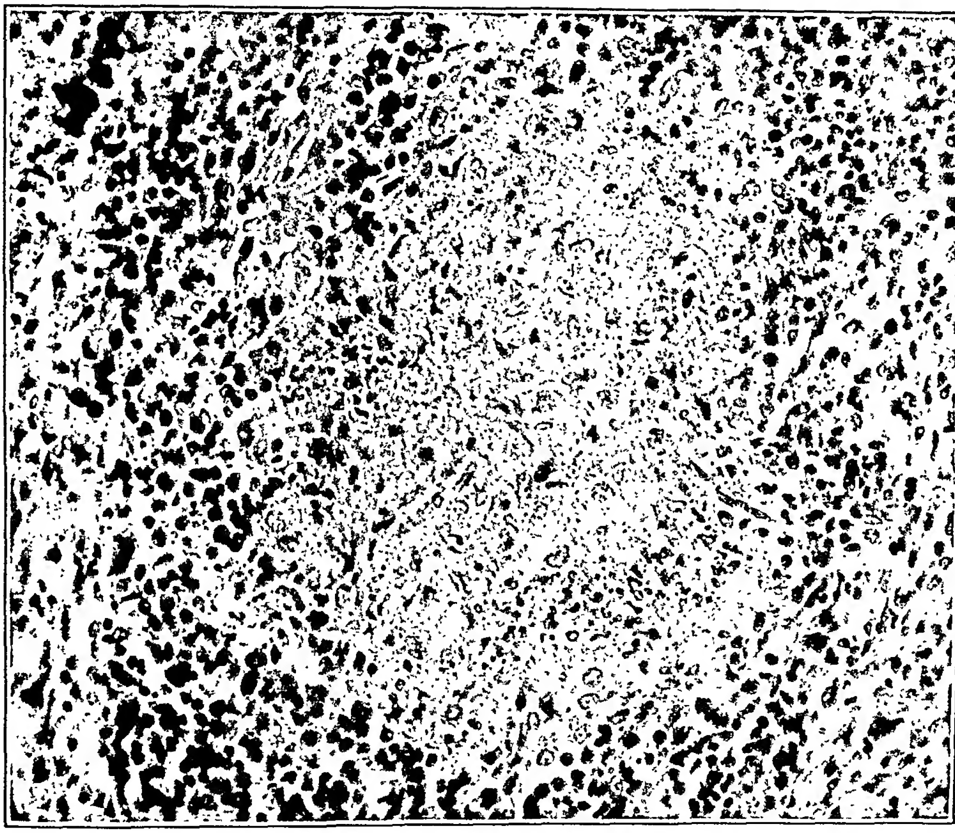

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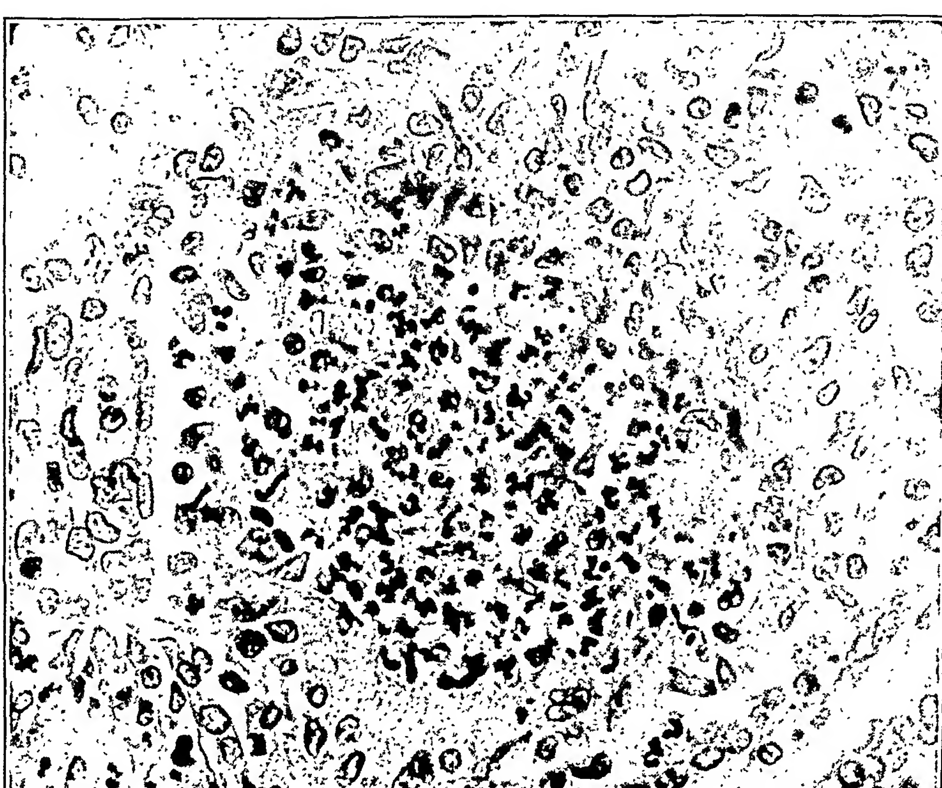

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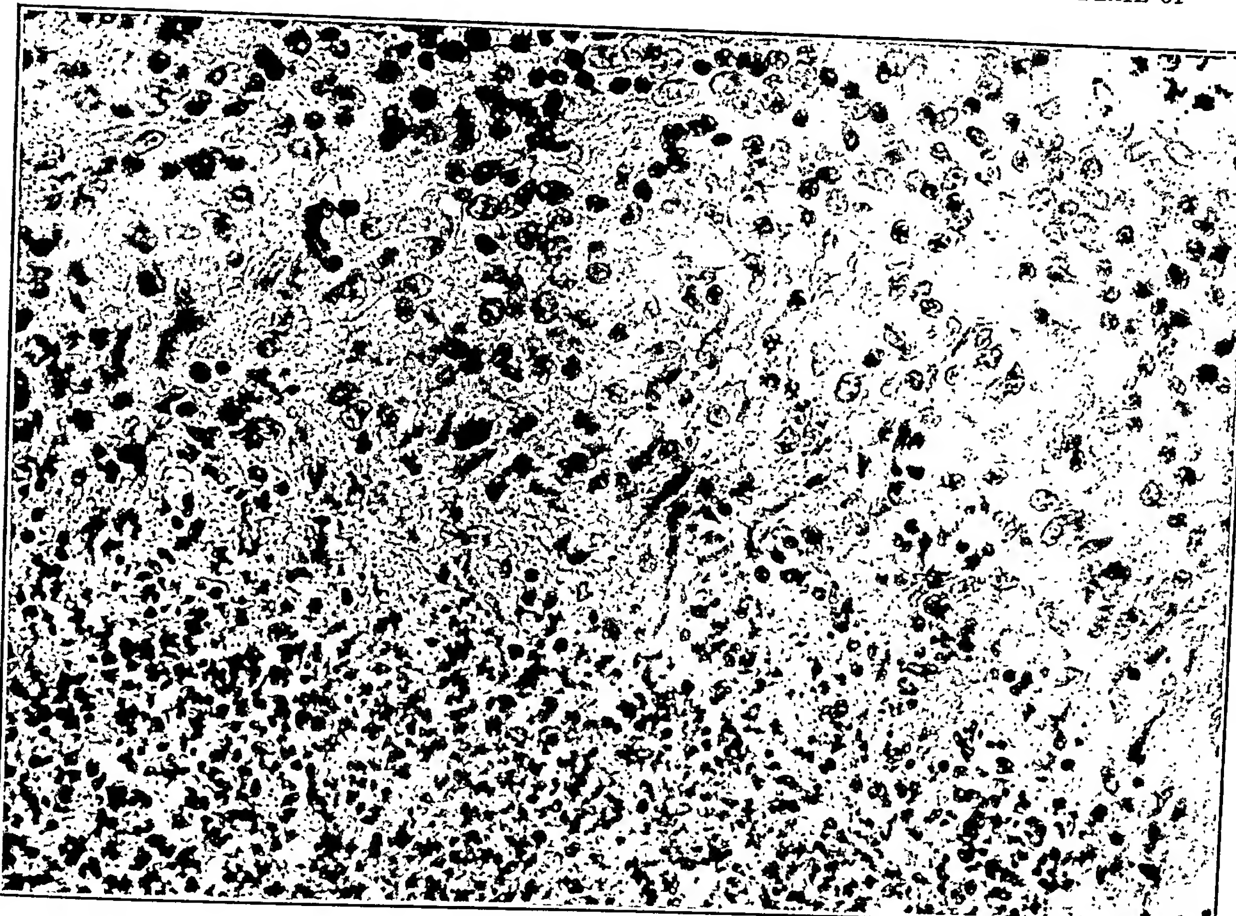

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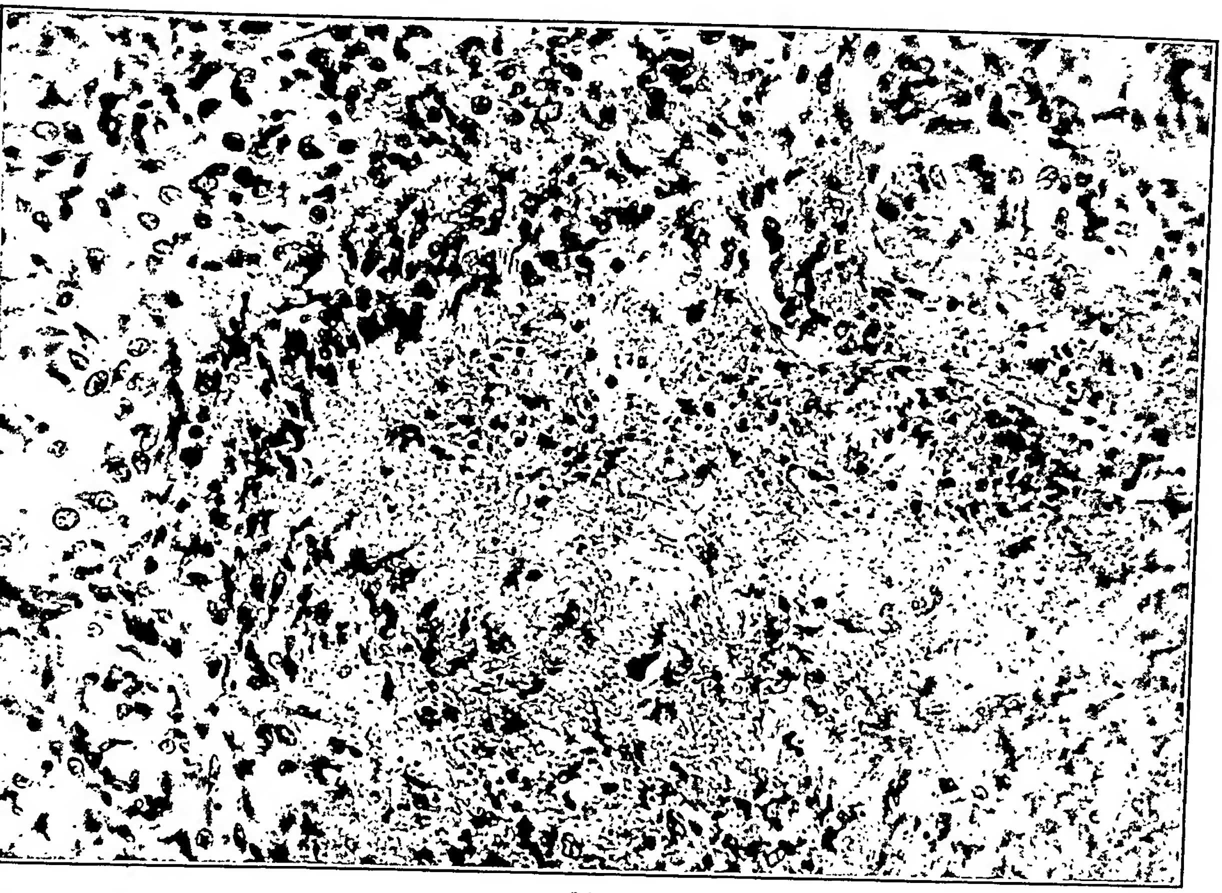

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GIANT CELLS AND THEIR RELATION TO CASEATION IN TUBERCULOSIS *

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The presence of giant cells, especially of the Langhans type, together with mononuclear tubercles is considered sufficient evidence in tissues to warrant the diagnosis of tuberculosis, even if tubercle bacilli are not demonstrated. Such cells are quite generally considered to be different from the ordinary foreign body giant cells and hence peculiar to tuberculosis. The frequent appearance of this cell in the midst of a tubercle, often occupying almost the whole of the structure, has led to the designation of such a lesion as a giant cell tubercle. To understand the significance of this cell mass in the tuberculous reaction, it would appear that two facts should be established. The first fact is the period in the tuberculous reaction in which the cell is produced, and the second is the mode of its formation and constitution.

The material which formed the basis of this study is a large number of serially sectioned tissues from guinea-pigs experimentally infected with human and bovine bacilli, fowl naturally infected with the avian bacillus and human tissues from necropsies and surgical operations. Serial sections were found of great value for two reasons. It was possible to examine the entire lesion and to reconstruct the giant cells, thus giving a more comprehensive idea of the size, shape and general characteristics of these cell masses.

Giant Cell Formation in the Guinea-Pig

Perhaps the most striking feature in the developing cellular reaction to the tubercle bacillus is the absence of giant cells. The next feature of prominence is the very common occurrence of caseation. From this it would appear that these cells are not essential in the process of caseation. This feature is of prime importance in the

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understanding of the rôle of this cell in the tuberculous reaction, for in the guinea-pig we have a host of high susceptibility to tubercle bacillus infection.

In the guinea-pig the mononuclear leucocyte plays the chief rôle in the production of the tubercle, the polymorphonuclear leucocyte in the production of caseation and the mononuclear leucocyte and lymphocyte in the disposing of the caseous material. It is in this later phase of the reaction, the appearance of caseation, that giant cells appear. I have not observed such cell formation in the earlier phases of the inflammatory reaction. When they do appear, one finds them in areas where the amount of necrosis or of caseation has been small and not in the areas where more extensive caseation has developed. It would seem that the ground substance or matrix of these cells is a small area of necrotic or caseous material. The peculiar arrangement of the nuclei in the periphery of the cell appears to be due to the immigration of the mononuclear leucocytes into the periphery of the dead tissue. One very commonly finds mononuclear leucocytes with markedly elongated nuclei, suggesting that they are on the point of entering the necrotic area. Lymphocytes are quite often found among the mononuclear leucocytes and they also appear in elongated form. Polymorphonuclear leucocytes are extremely rare in these areas. When mononuclear leucocytes accumulate in sufficient numbers, the picture of a giant cell tubercle is produced.

Giant Cell Formation in the Fowl

As the cases of avian tuberculosis were all natural infections, it was not possible to follow in time sequence the development of the tuberculous reaction. It was possible, however, to find numerous examples of the various stages as seen in the guinea-pig. Giant cells were far more numerous than in the guinea-pig. It was apparent that here we were dealing with a much better balance between the resistance of the host on the one hand and the virulence of the parasite on the other.

The same cell types react in the tuberculous process in the fowl that do in the guinea-pig in the formation of the tubercle and in caseation. It would appear that the giant cells would in all probability be formed in the same manner. In fact, the fowl tissues show much more strikingly the process of such cell formation than do the

tissues of the guinea-pig. Numerous examples of giant cell tubercles were found. In many instances it was possible to demonstrate the presence of cell exudate undergoing caseation in the center with large numbers of mononuclear leucocytes in the periphery of the caseating material. The intense eosin staining of the caseating mass makes a striking color contrast with the hematoxylin-staining mononuclear leucocytes surrounding it. Many of the isolated giant cells were roughly the size of mononuclear tubercles in the same field. Mononuclear leucocytes were numerous in the tissue adjacent to the caseating material and there was evidence that they wander into it. Mitoses in these cells were quite often found. Lymphocytes were also rather numerous among the mononuclear leucocytes. In the giant cell, where caseation has progressed to such a point that no nuclear fragments stain with hematoxylin, polymorphonuclear leucocytes were no longer found. In the earlier phase polymorphonuclear leucocytes were present among the mononuclear leucocytes at the periphery of and in the necrosing area.

Another finding of interest in the fowl tissues was the presence of from two to a dozen or more giant cells in close proximity to each other. In some of these areas there were bits of caseous material in the central portion which showed no nuclear content. It would seem that these areas were originally relatively small areas of caseation which for some reason have become broken up into smaller units. These units have then been surrounded and invaded by mononuclear leucocytes, thus forming giant cells. On tracing these areas through in serial sections, one found that many of these cell masses were continuous with an area of caseous material which might not contain any nuclear structures.

With an acid-fast stain large numbers of bacilli could be demonstrated in many of the giant cells. They occurred in colonies and in scattered groups, both in the necrotic area and in the nuclear zone. In these same cell masses there was present a small to a large number of clear spaces which corresponded in size, shape and distribution to the stained bacilli. In other giant cells no stained bacilli were found, but "phantoms" of bacillary forms were very commonly present.

Giant Cell Formation in the Human

It is possible to demonstrate in suitable human tissue all of the stages of the tuberculous reaction as seen in the guinea-pig or in the fowl. It seems logical, therefore, to expect that giant cell formation would occur in the same manner.

In the examination of old and of more recent tuberculous lesions in the same case one commonly finds giant cells in the former and not in the latter. From this it would appear that the formation of these cells is definitely associated with the more chronic lesions where a better balance between infection and resistance has been established.

These cells are but seldom found in close relation to large areas of caseation. They are found at a variable distance from the caseous mass and in this location one also finds giant cell tubercles.

As far as can be determined this process does not occur during the formation of the "epithelioid" tubercle. Neither does it appear to play any part in the process leading up to caseation. Apparently it does not occur in the tubercle unless necrosis or caseation supervenes. This is well illustrated in certain cases of hyperplastic tuberculosis where there is little evidence of necrosis or of caseation. In such cases giant cells are extremely rare. In other words, it seems that this cell is a late product in the tuberculous reaction, if it occurs at all.

If one studies a large number of giant cells, the variation in size, shape and nuclear content is very striking. The arrangement of the nuclei also shows great variation, although the peripheral arrangement is perhaps the most common. The substance which has been termed the cytoplasm of the cell mass apparently bears very little relation to the nuclear content, for one finds some cells having few nuclei with a larger amount of so-called cytoplasm than others which are heavily loaded with nuclei. By rough estimation of the volume of the cytoplasm of mononuclear leucocytes and of giant cells, computed from serial sections, one finds such great variation that it would seem highly improbable that the cytoplasm of the multiplied or accumulated mononuclear leucocytes alone constitutes the cytoplasm of the cell.

These cells vary from 30 to 300 microns in thickness. Reconstruction shows them very irregular in outline and more or less spheroid

or lenticular in shape. What appears to be two well formed and separate cells in one section often becomes a single larger cell a few sections farther on. This union and division may be present in more than one level. When reconstructed, some of these cells have the form of a capital letter H.

The distribution of the nuclei in the various levels of the giant cell is just as variable as the shape and size. The nuclei may be all through the cell at the center and around the periphery a few sections away, or the distribution may be reversed. The number of nuclei varies from less than 100 to more than 1,000 in the entire cell mass. Two types of nuclei are often present, those of the mononuclear leucocyte, which predominate, and those of the lymphocyte, which are present in small numbers. Both of these types of cell with markedly elongated nuclei can be found entering the cell mass. Very rarely a polymorphonuclear leucocyte is also seen in the giant cell.

In cases of pulmonary tuberculosis associated with a considerable degree of anthracosis in which the tuberculous process is of such a character that giant cells are common, it is quite often possible to find them containing anthracotic pigment. In such cells the pigment is peripherally arranged if the nuclei are so arranged and is scattered throughout the cell if the nuclei are thus placed. If the nuclei are peripherally arranged, the central portion of the cell is pigment-free. The amount of pigment varies from a few granules to an amount which, even under low power microscopic examination, gives the cell a distinct brownish black color. Instances have been observed in which the only pigment present has been in association with a single nucleus considerably separated from the major number of closely grouped nuclei in the cell. In the tissue surrounding the pigment-containing giant cells, pigment-laden mononuclear leucocytes are commonly encountered. From these observations it would seem fairly certain that the pigmentation of these cells has been brought about by the invasion of dead tissue by one to many pigment-containing mononuclear leucocytes.

These findings led to a more careful study of the substance which formed the matrix of the cell mass. It was found that the condition present in the human tissue corresponded to that observed in the guinea-pig. In other words, in order to have the formation of such cells brought about in human tuberculosis it is necessary to have

small areas of caseation, or of necrosis of the mononuclear leucocytes, produced at a previous date.

Since reticulum is a constant finding in a well formed "epithelioid" tubercle and since it appears that giant cells are formed in such tubercles after caseation or necrosis has supervened, it became of importance to determine the relation of reticulum to these cells. Tissues for this study were very kindly put at my disposal by Dr. William Snow Miller. This study revealed varying amounts of broken, jagged particles of disintegrating reticulum in practically all parts of the cell. In most places the cell was entirely separated from the surrounding well formed reticulum but in some areas these fibers extended into the cell mass for a short distance. The even texture of the viable reticulum stood out in sharp contrast to the jagged and granular bits of reticulum scattered haphazardly within the giant cell.

Discussion

A comprehensive review of the literature on giant cell formation will not be undertaken here. Suffice it to say that at the present time there are two theories. There is the original conception of Weigert that such cells are formed by the continued nuclear division, without division of the cytoplasm, of a single cell. This theory still has its ardent supporters, among whom are Cunningham, Sabin *et al.*[1] These authors would have this cell formed by continued amitotic division of the monocyte. The other theory is that the cell is formed by the fusion of many "epithelioid" cells. The most recent advocate of this theory is Maximow,[2] who would have the lymphocyte also participate in this fusion.

It is generally considered that giant cell formation precedes caseation and that it becomes involved in and destroyed by this process.

The findings presented above would seem to place in a different light the time and mode of production and the function of the so-called giant cell. It appears quite certain that this formation does not precede small areas of caseation or of necrosis. This would place the cell late in the tuberculous reaction, as far as the individual lesion is concerned. It would place such a cell in the reparative stage of the disease. Thus one can account for the inability to demonstrate tubercle bacilli in many of these cells.

Placing the giant cell in the reparative stage of the disease would

explain the finding of solitary giant cell tubercles in tissues which show no other evidence of tuberculous involvement. It coincides, in a way, with the statement of Smith [3] that "the occasional single giant cell in otherwise normal tissue of tuberculous cattle may be regarded as a tubercle in its simplest as well as most highly immune type." I do not consider this to be the simplest or the most highly immune type of tubercle, but rather a tubercle which has undergone necrosis or caseation, probably the latter, and is in a state of repair after the damage has been done. I believe the simplest and most highly immune type to be the mononuclear or "epithelioid" tubercle in which tubercle bacilli are destroyed without necrosis ensuing and without giant cell formation. The healed stage of such a tubercle would be, at most, a minute scar.

The finding of both lymphocytes and mononuclear leucocytes in the giant cell corresponds to the cellular reaction in the larger areas of caseation where both types of cell are found penetrating the caseous mass. It would appear that these larger areas of caseation prevent formation of giant cells because of their size and not because of any difference in cellular reaction. Definite evidence has been presented in the fowl, and the same picture on a smaller scale has been observed in the human and in the guinea-pig, that if the caseous material is broken up into smaller units, groups of giant cells will be produced. This evidence would suggest that a large number of these cells have caseous material as their matrix, and that this constitutes in large part the substance which has been regarded as the living cytoplasm of the cell. As caseation often takes place in the central portion of the tubercle, this would also account for the presence of such a cell in this location.

The finding of fragmented, jagged bits of reticulum and of anthracotic pigment in various parts of giant cells adds weight to the above evidence.

Further corroborative evidence is the finding of mononuclear leucocytes and lymphocytes wandering into the dead substance. Why the cells should not penetrate farther into the dead tissue in the case of the Langhans type of cell, is not clear. It may be due to lack of chemotaxis, but it would be more likely that the compactness of the material impedes further progress. The lack of compactness would explain why the invading cells are present in all portions of the dead tissue in some of the areas.

The theory of giant cell formation from a single cell seems to be incompatible with the evidence cited above. Whether the cells fuse after they wander into the dead tissue seems impossible of determination. Whether they do or do not fuse would appear to be of minor importance. The presence of lymphocytes in the cell can be explained on a basis other than that they are in process of being transformed into mononuclear leucocytes. No evidence is at hand, in the tissues examined, to suggest any such transformation.

The function of the giant cell, which from the above evidence appears not to be a true giant cell, apparently is to digest or remove in some manner the dead tissue. After this has been accomplished all evidence of the process left would be a scar which could no longer be identified as a lesion peculiar to tuberculosis.

It would seem that a true giant cell would be one where there is, when compared to a normal sized cell, a large amount of living cytoplasm with one or many living nuclei. Dead material would constitute no part of such a cell. Such giant cells are those seen in the most malignant types of neoplastic growths. With this as a standard, the cell masses in tuberculosis can hardly be looked upon as true giant cells.

Conclusions

Giant cell formation in avian, bovine and human tuberculosis appears to be brought about in a similar manner.

Giant cells are an indication of a reparative process in small areas of caseation or of simple necrosis of tissue — a reaction to a foreign body.

Giant cells in tuberculosis are not true giant cells. They are bits of dead inflammatory tissue which have been more or less completely surrounded and invaded by mononuclear leucocytes, less commonly by lymphocytes and very rarely by a polymorphonuclear leucocyte.

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2. Maximow, A. A. *J. Infect. Dis.*, 1925, xxxvii, 418.
3. Smith, T. *J. A. M. A.*, 1917, lxviii, 764.

DESCRIPTION OF PLATES

PLATES 62–65

FIG. 1. Guinea-pig spleen seven weeks after groin inoculation. Small area of caseation with four giant cells. Lesion in histologic unit of the pulp. x 350.

FIG. 2. Chicken spleen. Massing of mononuclear leucocytes around an area undergoing caseation. Numerous polymorphonuclear leucocytes in center still intact. x 350.

FIG. 3. Chicken spleen. An area similar to Fig. 2, except that the mass in the center has all undergone caseation. Note mitotic figures at about 6.15 and 7.30 o'clock. x 350.

FIG. 4. Turkey liver. A "nest" of giant cells. This represents an area of old caseous material which has broken up and in which the invasion of mononuclear leucocytes has caused giant cells to be formed. Note that the caseous material in the center is devoid of nuclei but otherwise resembles the cytoplasm of the giant cells. x 350.

FIG. 5. Two giant cells in human spleen. Both show lymphocytes as well as mononuclear leucocyte nuclei. At the lower right hand corner of the cell to the right there is a mononuclear leucocyte elongated in the process of entering the giant cell. x 200.

FIG. 6. Giant cell in human lung. The cytoplasm is composed of caseating material with much nuclear "dust" present. Adjacent to the mononuclear nuclei at the lower edge are a few anthracotic granules. x 350.

FIG. 7. Giant cell in human fallopian tube. Note lymphocytes in center and two elongated mononuclear leucocytes at the lower right hand corner. x 500.

FIG. 8. Giant cell in human lung. Note anthracotic pigment scattered in region of nuclei. Also individual anthracotic-laden mononuclear leucocytes in the tissue above and below the giant cell. x 350.

FIG. 9. An area of caseation in a human lymph node. Two well formed giant cells at the left and one forming at the right. Mononuclear leucocytes and lymphocytes are invading the area in the center. x 200.

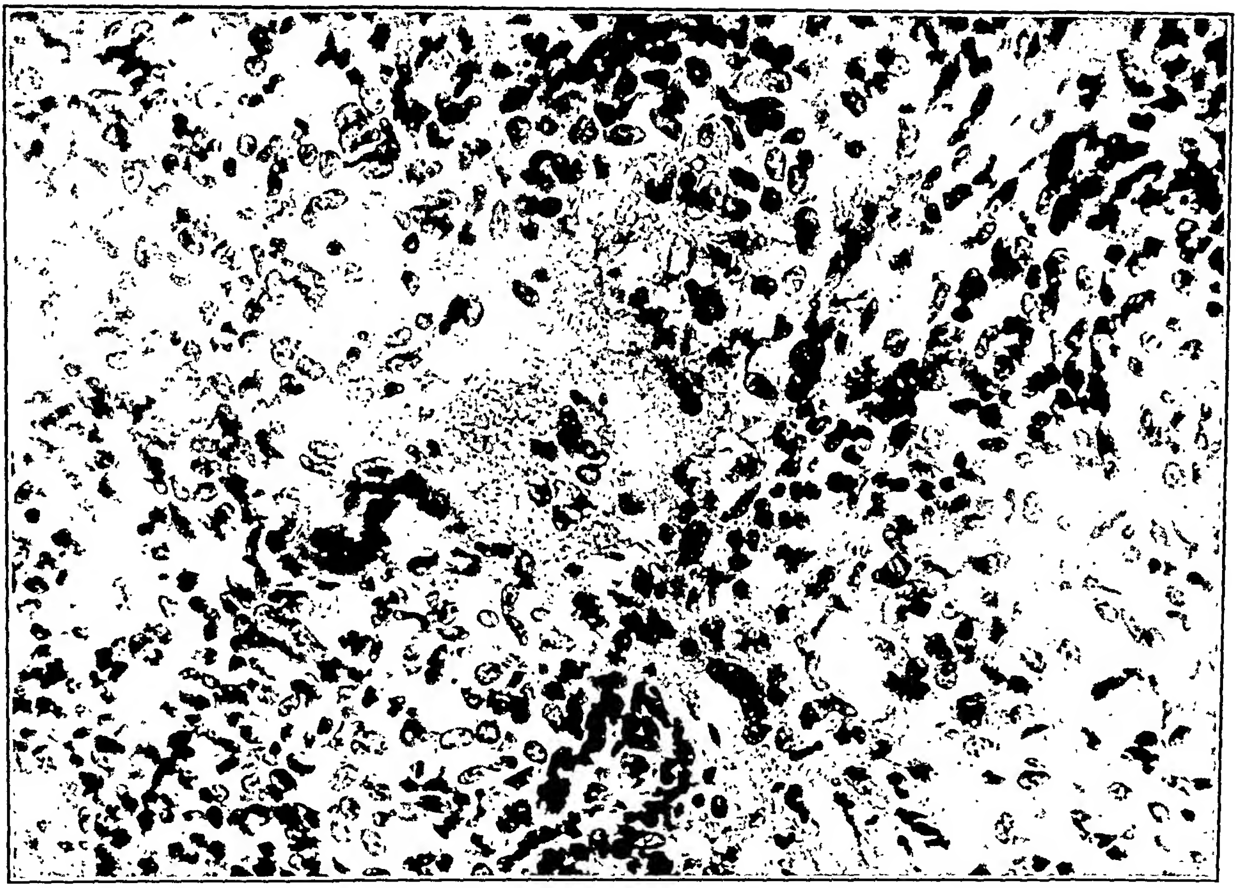
FIG. 10. Giant cell tubercle in human kidney. Note the arrangement of the nuclei about four centers. x 200.

FIG. 11. Human lung showing giant cells in various stages of formation. x 100.

FIG. 12. Giant cell at right of Fig. 11. Reticulum stain. Note the small fragments of reticulum in the center. x 500.

FIG. 13. Human lung. Reticulum stain. Mononuclear tubercle with basketwork of reticulum and no necrosis below. Compare size of giant cell above with tubercle below. Note separation of reticulum from giant cell at its periphery and the particles of jagged reticulum within the giant cell. x 500.

FIG. 14. Human lung. Reticulum stain. Giant cells showing broken reticulum within them and separation from surrounding viable reticulum. x 500.



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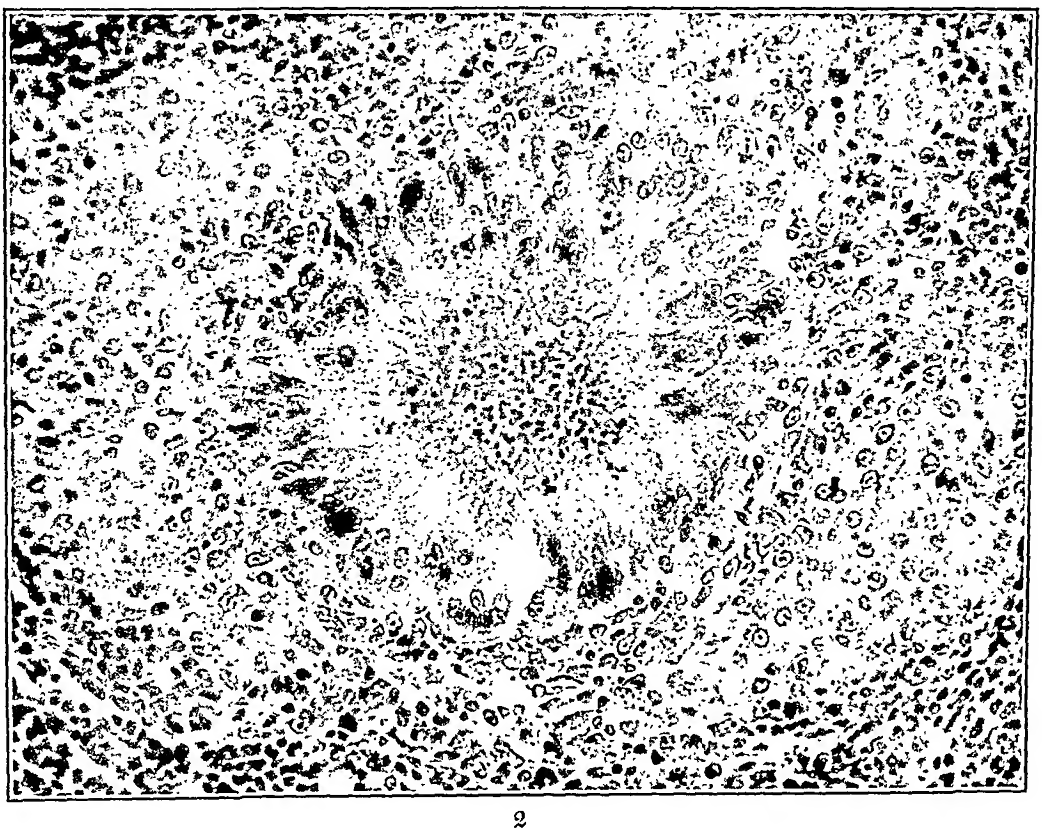

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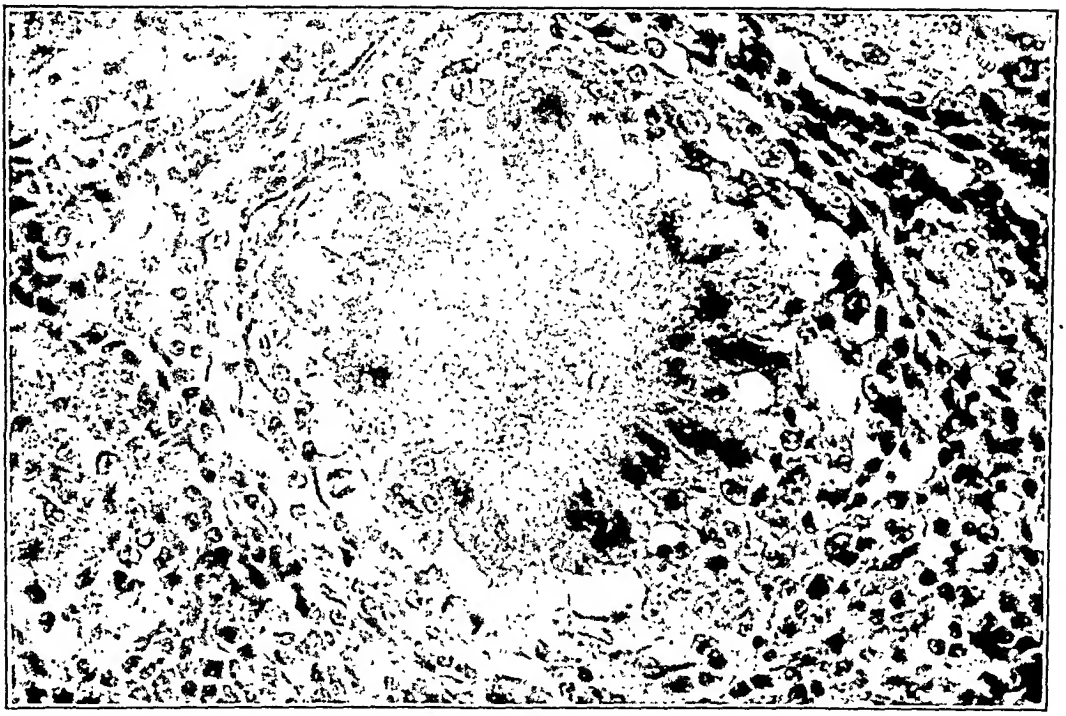
Medlar Giant Cells and Their Relation to Caseation



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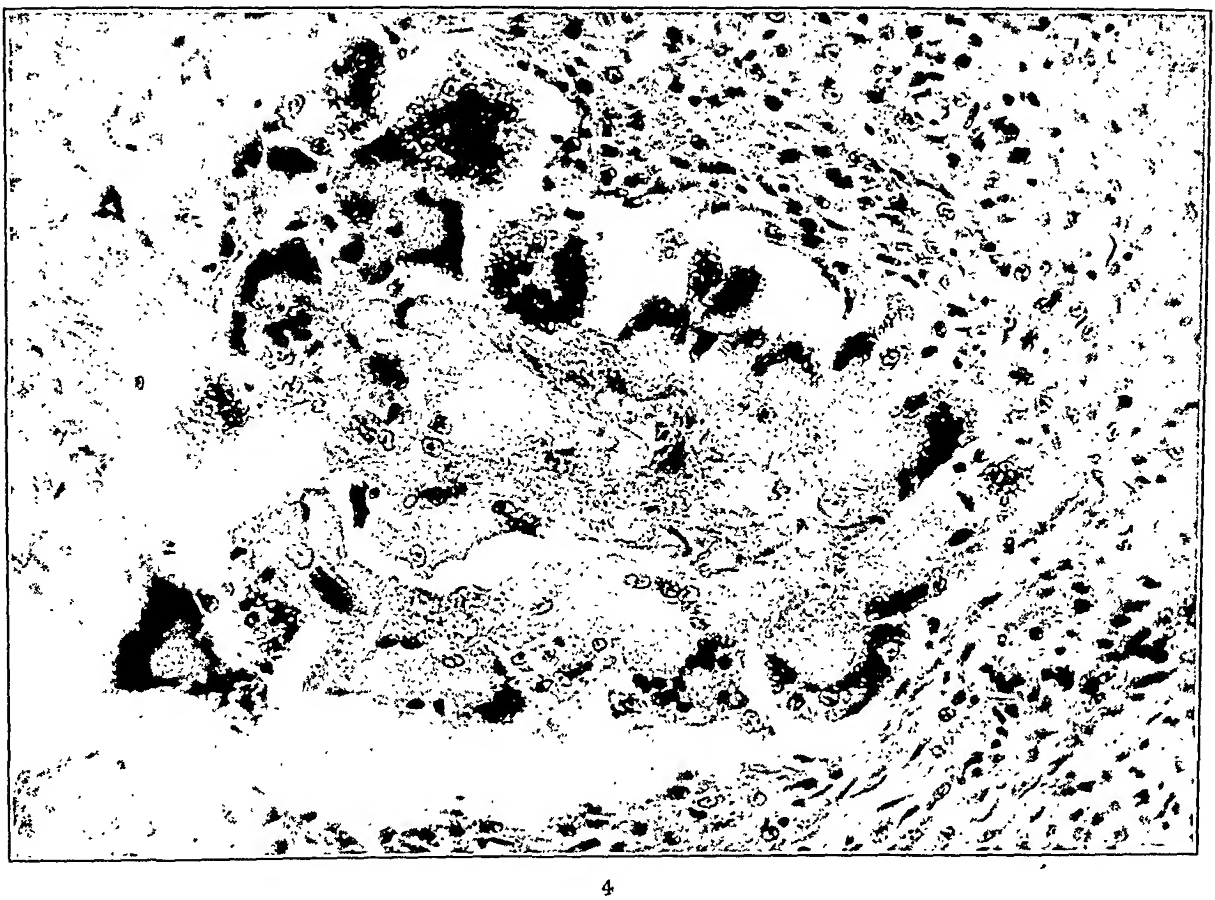

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Medlar Giant Cells and Their Relation to Caseation

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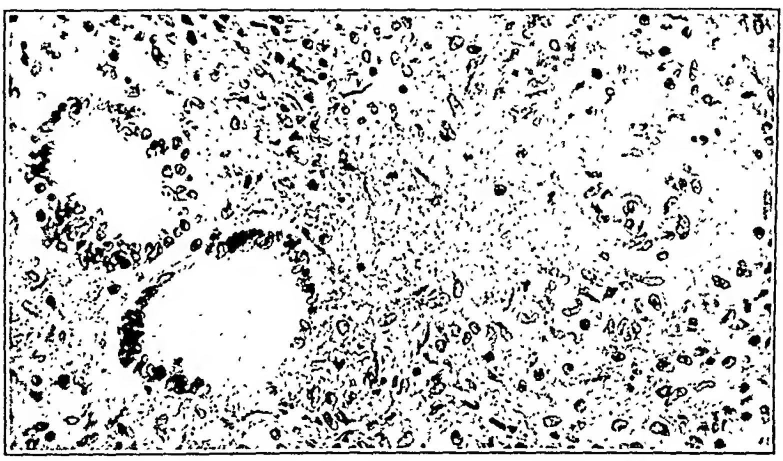

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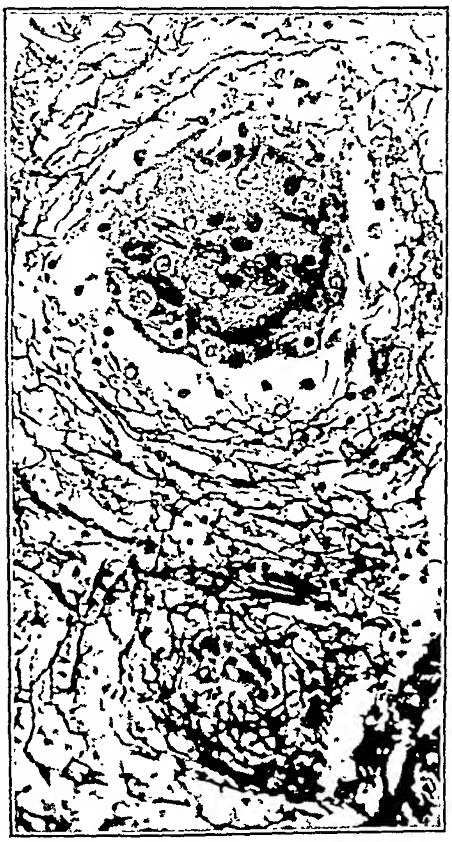

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Medlar

Giant Cells and Their Relation to Caseation

AUTOTRANSPLANTATION AND HOMOIOTRANSPLANTATION OF THE THYROID GLAND IN THE RAT

WITH SOME OBSERVATIONS ON TRANSPLANTATION OF THE PARATHYROID, UTERUS AND OVARIES *

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In former papers we compared auto- and homoiotransplantation of thyroid and other tissues in the guinea-pig. It was then of interest to determine how far the conclusions we arrived at in the case of the guinea-pig hold good also in the case of other animals and how far we have to deal with reaction types that apply generally. For purposes of comparison, therefore, we carried out auto- and homoiotransplantations in the rat. On a former occasion we had already compared multiple transplantations of organ pieces in the rat under conditions of homoio- and syngenesiotransplantation.[1]

A. AUTOTRANSPLANTATION

In the first two weeks following transplantation, conditions are essentially the same as in autotransplantation of thyroid in the guinea-pig; there is a ring of preserved thyroid tissue surrounding a central necrotic zone. The latter is smaller in the rat than in the guinea-pig, because the transplanted lobe of thyroid is smaller in this case. Blood vessels and connective tissue cells are attracted by necrotic material in which is found the colloid of necrotic acini. The myxoid or edematous zone in the periphery of the organized center, which we observed in the guinea-pig, does not occur in the rat, although the center is here also well supplied with vessels and some loose connective tissue may occur in it. In the rat, the organized center is composed of wavy fibrillar connective tissue arranged in parallel bundles separated by clefts, which possibly represent lymph spaces. There are large capillaries here also and some of the original transplanted arteries which probably serve as channels for the new vessels. On the whole, the organization progresses rapidly and it is

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usually completed by the fifteenth day. Lymphocytes appear in small numbers in the central or in the peripheral connective tissue; they may also be found in some lymph vessels. The peripheral part of the parathyroid is also preserved, but its center is replaced by connective tissue.

The ring of thyroid tissue consists of acini which adjoin each other closely. Mitoses in acinus cells occur. In the first week after transplantation the colloid may be lacking or be incomplete in some acini; phagocytes may contribute to its destruction. Toward the end of this period the colloid has usually reassumed its solid character and it is retracted; the epithelium is usually lower than in the remnant of the original, not transplanted thyroid. On the whole, the number of fibroblasts which migrate between the acini toward the center of the transplant is very moderate, and the collection of lymphocytes which may occur is very small. Both are caused by conditions which are independent of the relationship between transplant and host; in particular, the stimulation of fibroblasts is caused by the necrotic material in the center of the thyroid. This or other non-specific factors here may also cause the slight increase in lymphocytes which is occasionally observed. Sometimes we can observe that the lymphocytes collect around some foreign bodies which are accidentally introduced into the subcutaneous pocket made for the transplantation.

A change of importance in the autotransplantation takes place usually around the fourth week, but occasionally somewhat later; at this time the thyroid ring becomes transformed into a curved structure of thyroid tissue which often has a serpentine form. Such an alteration is perhaps brought about through the opening of the ring of thyroid tissue and the extrusion of the central connective tissue into the neighboring tissue or perhaps through absorption of the central fibrous tissue as the result of the activity of fibroblasts. The connective tissue around the thyroid may be partly hyaline in character; this may possibly be due to the organization of colloid material in the necrotic center of the transplant. There is a good supply of capillaries around the acini; but only occasionally are they surrounded by strands of fibroblasts. The acini are well formed and show low epithelium. Toward the end of the fifth week, lymphocytes may be lacking or be present only in very small collections in the transplant.

Between the fortieth and sixtieth day, the transplant begins to

resemble the normal thyroid, but the epithelium is usually lower than in the remnants of the original gland from which the pieces were removed and a very small amount of fibrous tissue may be found between some of the acini. There are no lymphocytes present or only a few in the lymph vessels.

If we compare the autotransplantation of thyroid in the rat with that in the guinea-pig, we notice their essential similarity. In both cases the ingrowth of fibroblasts is very restricted and apparently is caused in the main by the presence of necrotic material in the center of the transplants. There is also the same tendency toward the formation of loose connective tissue in the center adjoining the thyroid ring, but this is more pronounced in the guinea-pig than in the rat. In both animals the central connective tissue is eliminated after some time; though how far an actual solution of this fibrous material takes place or what part is played by the pushing of the fibrous tissue to the outside of the transplant is uncertain. Furthermore, in both the guinea-pig and the rat the vessels are attracted by the thyroid transplant which gives off substances of an "auto" character and in both cases the lymphocytic collection which may be observed is very restricted and non-specific, caused by slight disturbances connected with the process of transplantation or by the accidental presence of foreign bodies. In autotransplantation of thyroid, therefore, in these animals the original injury to the thyroid tissue, with destruction of colloid in acini, is gradually repaired, and the transplant assumes more and more the character of the normal gland, although the epithelium of the acini becomes on the whole lower than in the autochthonous gland.

B. Homoiotransplantation

We carried out three series of homoiotransplantation of the thyroid in the rat. In the first series donor and host had been obtained from different cities; we were therefore justified in our conclusion that they were not related to each other. In a second series of white rats host and donor were also usually not related to each other, but we could not in some cases exclude a distant relationship with the same certainty as in the first series. In a third series of transplantations, host and donor had different color patterns and had also been received from different cities. In describing our results, we shall ex-

tend the use of grades which we employed in a former paper dealing with homoiotransplantation of the thyroid in the guinea-pig. However, this grading can be used only at a period following transplantation when the reaction is fairly definitely developed, beginning with the eighteenth day. Tentatively it may also be used at somewhat earlier periods following transplantation. We had previously made use of such a system of grades in analyzing compensatory hypertrophy in the thyroid of the guinea-pig.

We shall first compare the results in Series I and II. Twenty-nine transplantations were used in the first series, and seventy-five transplantations in the second series. Thirteen transplantations constitute the third series. In the early period up to the eighth day following transplantation, about two or three rows of acini, or sometimes less, remain preserved. The acini are small and colloid has been lost in many of them, but it is preserved in others. Through the thyroid ring, especially through interstices, capillaries grow toward the center; some of them are quite large at this period. Mitoses may be seen in acinus cells. Toward the end of this period the necrotic center of the transplant is in some cases organized by not very dense small-celled connective tissue or sometimes by dense connective tissue; in other cases some necrotic material may still be left. It is taken up and removed, at least a part of it, by phagocytes. Hemorrhages may also occur from the newly formed capillaries which penetrate into the center. From the sixth day on, lymphocytes appear in some cases, at first in lymph vessels which penetrate through the thyroid ring; toward the end of this period they may begin to collect at the periphery of the center and may even fill the center diffusely; connective tissue as well as some lymphocytes also occasionally surround some acini. The peripheral part of the parathyroid may also be preserved, while the center is necrotic and in process of organization. Mitoses occur in the parathyroid as well as in thyroid cells. There is no noticeable difference between the two series at this time.

In Series I from ten to fifteen days after transplantation, the "homoio" character of the transplant is usually pronounced, but certain variations occur. Sometimes no thyroid tissue is found, but only fibrous tissue with lymphocytes. In most cases the lymphocytic infiltration is marked and in some instances so intense that the appearance of lymph glands is simulated. Surrounded by masses of

lymphocytes there are some isolated acini or bundles of acini still visible. In other transplants the lymphocytes penetrate between and into the acini and destroy them. The center is usually converted into fibrous tissue, the amount of fibrous tissue present being much greater and the fibrous character much more pronounced than in the case of autotransplantation. In the beginning of this period, the organization of the central necrotic material is not yet complete in every case. The blood vessels in the center are no longer very conspicuous in the second half of this period. The thyroid ring when present is incomplete. However, in one case the transplant was better preserved and the central connective tissue smaller in quantity, so that the piece approached in certain respects the structure of an autotransplant except that the lymphocytic infiltration was much more pronounced. There seems to be some indication that the homoio-reaction is especially marked after transplantation in certain strains of rats.

At sixteen and seventeen days, we find again some variation in the condition of the homoiotransplants of the first series, especially as far as the amount of preserved thyroid tissue is concerned. In some cases it is considerable but in other cases very slight. In the latter condition the colloid is lost in the majority of the remaining acini. Fibrous bands separate the acini or bundles of acini. Lymphocytes also penetrate into the thyroid tissue and separate acini in certain areas. The centers of the transplants as well as the lymph vessels are filled with lymphocytes.

In Series II, conditions are somewhat similar from the tenth to the twelfth day. However, in general the thyroid ring is somewhat better preserved in this series. At ten days the center is not yet completely organized in every case. Connective tissue and lymphocytes surround some acini. At twelve days there is noticeable for the first time a considerable lymphocytic infiltration. If we compare the transplants of the first and second series between the thirteenth and eighteenth day, we find in both a similar condition, but the average preservation is slightly better in Series II. In both the first and second groups the preservation at this time is better than at the later period, from twenty to thirty days. The latter period seems to represent the critical time in which the destruction has reached its maximum. At the thirteenth day, the center in Series II is usually organized by fibrous tissue but some loose con-

nective tissue is also occasionally found there. The colloid may be preserved or partly destroyed; in the peripheral acini especially it is often preserved. Fibrous bands surround the acini. There is moderate or marked lymphocytic infiltration in the center as well as in the periphery of the transplant and also between the acini. The parathyroid is likewise much infiltrated with lymphocytes. At fifteen days, there is found in one transplant a condition resembling that of a syngenesiotransplant: loose connective tissue, fat tissue and blood vessels are seen in the center; there is slight lymphocytic infiltration in the capsule of the thyroid and from here lymphocytes penetrate in places between the acini; also some lymphocytic infiltration is found in the center of the transplant; mitoses occur in thyroid as well as in parathyroid cells. But in other specimens at this period, lymphocytes and connective tissue have destroyed the greater part or the whole of the thyroid, and connective tissue surrounds and separates the remaining acini from each other. At seventeen days, there is usually much lymphocytic infiltration in the periphery of the transplant, mainly around the vessels. The fibrous center is large and wide vessels pass toward it through the thyroid ring. Lymph vessels are filled with lymphocytes and there is some indication that lymphocytes can migrate into the lumen of the vessel from the surrounding tissue. Lymphocytes also enter the parathyroid; however, the lymphocytic infiltration varies in amount in different cases. Occasionally mitoses are still found in acinus cells.

In order to obtain a quantitative measure of the strength of the homoio-reaction we arranged the various transplants in six classes as follows: Grade 6 corresponds to an autotransplant. Grade 5 is similar to a syngenesiotransplant; the structure of the transplant may resemble that of an autotransplant or the amount of connective tissue in the center may be slightly increased; but there is a definite lymphocytic infiltration noticeable. In grade 4 there is a well preserved thyroid ring, but there is an increase in fibrous tissue around the acini and the lymphocytic infiltration is quite marked in places. The center is fibrous and enlarged. In grade 3 there is still a thyroid ring present, but it is thin and may be not quite complete or there are at least larger bundles of acini preserved. The increase of fibrous tissue surrounding the acini is very pronounced and the lymphocytic infiltration is also marked. The center consists likewise of dense fibrous tissue which is present in a relatively considerable

quantity. In grade 2 only isolated acini or bundles of acini are found in the fibrous tissue. The lymphocytic infiltration is quite marked. In grade 1 the thyroid proper has been entirely destroyed and only fibrous tissue and some lymphocytes are found.

If we use this classification, we find in the period between the thirteenth and eighteenth day in Series I an average of grade 3 and in Series II of 3.4. That means that the preservation of the transplant is slightly better in Series II.

SERIES I. 19 TO 40 DAYS. In this period after transplantation there are in Series I thirteen experiments with an average grade of 2.5, while in Series II there are twenty-seven experiments with an average grade of 2.7. Between twenty and thirty days the maximum in the homoio-reaction is obtained; the destruction of the transplants is greatest.

The condition found in Series I is indicated by the following grades. *20 days:* (a) grade 1, much lymphocytic reaction. (*b*) grade 1. (*c*) grade 4, thyroid ring, in places dense lymphocytic infiltration; in other places the lymphocytic infiltration not dense; most acini without colloid, some with colloid. (*d*) grade 1, lymphocytes present. (*e*) grade 3 or 3–4; there are one or two rows of thyroid acini; acini separated by much fibrous tissue and much lymphocytic infiltration; colloid lacking in acini. (*f*) grade 2; there are in the fibrous tissue some bundles of compressed acini the lumina of which are small slits; intense lymphocytic infiltration. *21 days:* grade 3, thyroid ring, acini with solid colloid or compressed slits; fibrous tissue with much lymphocytic infiltration surrounding acini. *24 days:* grade 2. *25 days:* grade 1. *30 days:* (*a*) grade 2–3; in periphery remnants of large and small acini with colloid; very dense lymphocytic infiltration. (*b*) grade 1 or 1–2. *40 days:* (*a*) grade 1. (*b*) grade 1. (*c*) grade 5, syngenesio-reaction, auto-structure with some masses of lymphocytes. (*d*) grade 5. The number of the experiments at different periods is relatively small in this series, but there is some indication that at forty days in some cases a recovery may have taken place in the condition of the homoiotransplants.

SERIES II. 19 TO 40 DAYS. This impression becomes more definite if we compare in Series II the grades of preservation and lymphocytic and connective tissue reaction in the periods from nineteen to thirty days with those in the periods between thirty and forty days.

At 19 days: grades 2, 2–3, 2–3, 2–3, 3, 1, 1. There is as a rule only a small amount of thyroid tissue preserved. The lymphocytic and connective tissue reaction is usually pronounced; lymphocytes as well as connective tissue help to destroy acini which are quite commonly without colloid. Lymphocytes separate and penetrate actively into acini. However, in some cases the fibrous tissue reaction is more pronounced than the lymphocytic invasion. On the whole the parathyroid, after its central part has once been organized, is usually less invaded and injured by connective tissue and lymphocytes than the thyroid tissue, but lymphocytes and connective tissue may also enter the parathyroid and partly destroy it.

At 20 and 21 days: grades 5, 2, 1. In one case, only fibrous tissue with some lymphocytes is found (grade 1). In the second case (grade 2), the transplant appears almost like a lymph gland. The large majority of the acini are destroyed, but a number of acini are still preserved and some of these contain colloid. Lymphocytic masses penetrate into the remaining acini from the outside as well as from the inside of the thyroid ring and thus help to destroy them. In one case (grade 5), the structure of the transplant approaches that of an autotransplant; it has the form of an ellipsoid. There is a small amount of loose areolar connective tissue in the center. In addition to some diffuse lymphocytic infiltration there are in places small collections of lymphocytes around vessels. In this case we have to deal with a typical syngenesio-reaction.

At 23 to 25 days: grade 2; only some islands of acini left; some acini still with colloid; almost all of the thyroid has been replaced by lymphocytic masses. Grades 1, 3, 1, 1, 2–3; acini without colloid form small clefts in the fibrous tissue; they are individually surrounded by fibrous tissue. There is marked lymphocytic infiltration. Less than one-half of the periphery still contains some acini. The peripheral part of the parathyroid is preserved.

At 30 days: grades 1, 2, 3; some parts of thyroid ring left; some acini with, some without colloid. There is an intense diffuse lymphocytic infiltration. Lymphocytes penetrate between the acini; bundles of acini, lying close together, are surrounded by lymphocytes. The center is filled with lymphocytes. Grade 2–3, only a small part of thyroid preserved; still some acini lying close together, with or without colloid. The majority of the acini are compressed and are without colloid; they are surrounded by fibrous tissue. In

some places, a very marked lymphocytic infiltration; in other places only few lymphocytes enter, disorganize and destroy acini. The surrounding connective tissue is very fibrous. Grade 4, rather well preserved ring of acini; the majority of acini are without colloid, some are with colloid. Fairly marked lymphocytic infiltration. A few lymphocytes are in periphery; the majority are in the center. Some fibrous bands around acini.

At 31 to 40 days: grade 5; auto-structure: ellipsoid of thyroid, acini close together. A little fibrous tissue and a very extensive lymphocytic infiltration in center. In several places in periphery collection of lymphocytes. Some lymphocytes penetrate between acini at certain points. Well preserved parathyroid with a number of mitoses in parathyroid cells; beginning lymphocytic infiltration in periphery of parathyroid. Grade 6, about like autotransplant; acini close together with good retracted colloid; in center areolar and some fibrous tissue, and also some blood pigment. Grade 5, in this case especially around parathyroid marked lymphocytic infiltration. Grade 4–5, very large and well preserved ring of acini, but increased amount of fibrous tissue in center. There is a slight diffuse and more severe localized lymphocytic infiltration, especially around parathyroid. Lymphocytes penetrate also a little between acini, but there is no destruction of acini. Grade 5, only in places in center small collections of lymphocytes. Grade 3–4, a number of follicles with colloid, some compressed acini. Much fibrous tissue in center. Intense infiltration with lymphocytes; in periphery collection of lymphocytes. Some parts of thyroid almost completely replaced by connective tissue.

We see in this series a distinct improvement in the preservation of the transplants and a diminution in the intensity of the lymphocytic reaction between the thirtieth and fortieth day. This improvement is maintained between the fortieth and eighty-fifth day after transplantation.

At 40 to 85 days: These experiments were carried out with animals of Series II. In two animals after forty-two days only epidermal cysts are found; they are derived from the strands of stratified epithelium which are admixed to the thyroid and parathyroid glands in the guinea-pig as well as in the rat. The grades in the other cases were as follows: *42 days:* grade 5, well formed acini with colloid; little connective tissue, some blood pigment in center. In certain

places, especially at both poles of the transplant and between acini, some lymphocytic infiltration; grade 5. *47 days:* grade 5; both similar to first specimen; in latter specimen only small parts of thyroid destroyed by lymphocytes. *49 days:* grade 3.5, a considerable number of well preserved acini; some with, but the majority without colloid. Large fibrous center. Intense lymphocytic infiltration. Lymphocytes invade and destroy many acini; lymph vessels are filled with lymphocytes. Also epithelial cyst is infiltrated with lymphocytes. Considerable part of thyroid is destroyed. *50 days:* grade 5; auto-structure, but in various places in center and also in periphery of transplant collections of lymphocytes, especially around blood vessels; they penetrate also a little between acini. Lymph vessels in center and in periphery filled with lymphocytes. *54 days:* grade 4; fairly good thyroid ring; at both poles lymphocytic masses. Around vessels in center and between acini considerable lymphocytic infiltration. *85 days:* grade 5. This experiment, in which donor and host were obtained from different cities, does not strictly belong to this series. We find auto-structure, but in several places masses of lymphocytes in lymph vessels and in connective tissue are seen and they begin to invade the thyroid. Grade 5.5; auto-structure; good colloid in acini. Around one lymph vessel in center collection of lymphocytes. The average grade of this series is 4.82.

We see thus, after the maximum of destruction has been reached in the course of the fourth week following transplantation, a rise in preservation of thyroid and a diminution of destruction of thyroid by lymphocytes. In evaluating these experiments we must consider that the experiments with the longest duration were carried out with animals of Series II, in which a relationship between donor and host could not be excluded with the same certainty as in Series I. It is therefore possible that a closer relationship between the animals may in part explain the result. On the other hand certain facts suggest the possibility of a gradual adaptation occurring between the transplanted tissue and tissues of the host. How far the one factor and how far the other two factors come into play, has to be investigated in further experiments, and these are being carried out at the present time.

Series III. In this series we transplanted the thyroid from white rats into two kinds of pure colored strains, a cream and a hooded strain which had been bred by Dr. Helen D. King, and which we

received through her kindness from the Wistar Institute. One transplant was examined after seven days, all the others were examined after twenty and twenty-one days.

After 7 days: From HaO white rat to a cream rat. There is a marked homoio-reaction. The acini are mostly without colloid, but some are with colloid. There is much connective tissue growing between the acini and consequently many acini are compressed and have a very small lumen; there is also a distinct connective tissue capsule around the transplant with definite lymphocytic infiltration. Collections of lymphocytes are found in lymph vessels which together with connective tissue enter the transplant.

All the other pieces were examined *twenty and twenty-one days* after transplantation. I. From HaO white rats into cream rats. (*a*) grade 1. (*b*) grade 1–2; fibrous tissue nodule with moderate lymphocytic infiltration; a few acini without colloid left, but in process of destruction; the large majority of the acini have been destroyed. Moderate lymphocytic infiltration in connective tissue; some lymphocytes invade acini. Connective tissue is still in process of organizing necrotic parts of the transplant. (*c*) grade 1. (*d*) grade 1–2; loose connective tissue with blood pigment in the center. Marked lymphocytic infiltration around some remnants of compressed acini which are being invaded and destroyed by lymphocytes. (*e*) grade 1. (*f*) grade 1; fibrous tissue with dense lymphocytic infiltration.

II. From white Micha rats to hooded rats. (*a*) grade 1. (*b*) grade 1. (*c*) grade 1; fibrous tissue and lymphocytic infiltration. (*d*) grade 1. (*e*) grade 1; dense lymphocytic infiltration. (*f*) grade 1.

As early as seven days after transplantation there is a very marked homoio-reaction noticeable in this series. There is much fibrous tissue formation around the transplant and between the acini. The acini are compressed and there is a distinct lymphocytic infiltration. As a result of these processes which evidently continue to act during the second and third week, we find twenty to twenty-one days after transplantation in ten cases a complete destruction of thyroid tissue (grade 1), while in only two cases small remnants of thyroid are left; these are very seriously injured as a result of the action of connective tissue and lymphocytes and they are near the point of complete destruction (grade 1–2). The reaction of the host against the transplant is therefore decidedly more marked and successful in case of exchange of organs between white and cream or

hooded rats than in cases in which organs were exchanged between non-related white rats. There is no essential difference between the action of cream and of hooded rats on thyroid transplants of white rats. The reaction is possibly slightly more marked in the case of the hooded rats.

Homoiotransplantation of Uterus and Ovaries in White Rats

In five animals, pieces of uterus, with or without ovaries, were homoiotransplanted into animals in which also the thyroid from the donor had been transplanted. The examination was carried out fifteen to twenty-three days after transplantation. In the thyroid transplant there has been in each case a very marked homoio-reaction; either the lymphocytic reaction has been very pronounced or the thyroid has been entirely destroyed. The transplanted uterus has also been partly destroyed; there is either lymphocytic infiltration in the transplanted muscle tissue and epithelium or at least in the adjoining fat tissue.

Summary

1. In all essential respects the laws governing autotransplantation of thyroid and parathyroid in the rat are the same as those established in the case of the guinea-pig. Connective tissue seems in both cases to be attracted by necrotic material; slight collections of lymphocytes are due to non-specific conditions, such as accidental presence of foreign bodies. In both cases, a gradual elimination of these abnormalities takes place and the autotransplant more and more resembles the autochthonous organ. Some differences exist between the methods of regulation which serve to eliminate the adverse conditions in the rat and guinea-pig, but these differences are due to factors of secondary importance, such as the size of the transplant.

2. After homoiotransplantation of the thyroid and parathyroid, the same agencies of attack on the part of the host come into play in the rat as in the guinea-pig; furthermore the intensity of this reaction seems to be approximately the same in the two animals.

3. The intensity of the aggressive reaction depends upon the relationship of host and donor. We carried out three series of trans-

plantations in which the grade of relationship varied. In the third series in which we transplanted tissues from white rats to pure strains of hooded and cream rats respectively, the intensity of the homoio-reaction was greatest. There was a slight difference only between the two other series in both of which white rats served both as donors and hosts. The reaction was here slightly more intense in the first series, where a distant relationship between donor and host could be excluded with greater certainty than in the second series.

4. In our second series in which a remote relationship between donor and host could not be excluded with the same certainty as in the other series, a maximum of destruction was observed during the fourth week after transplantation. From then on, an improvement in the condition of the transplant was observed. How far accidental relationship determines this result, how far a gradual adaptation between host and transplant is responsible for it, have to be determined in further experiments.

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AUTOTRANSPLANTATION AND HOMOIOTRANSPLANTATION OF CARTILAGE AND BONE IN THE RAT*

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In the preceding paper we have studied the auto- and homoiotransplantation of thyroid and parathyroid in the rat and compared the results with those obtained previously in the guinea-pig. We used three different groups of rats in Series I, II and III and in particular we tested the effect of a still more distant relationship between host and donor by transplanting the thyroid from white rats to pure strains of colored rats. In this communication we shall discuss the fate of cartilage of three different groups of rats. In the majority of cases we transplanted thyroid and cartilage into the same individual. We are thus in a position to compare in an exact manner the action of homoio-toxins of various strengths on the cartilage with that on the thyroid. We furthermore made some observations on normal xiphoid cartilage and on cartilage after autotransplantation in the rat.

The normal, non-transplanted xiphoid cartilage in the rat is surrounded by fat tissue in which the connective tissue septa are fine. At a certain level, muscle tissue attaches itself to the perichondrium. The width of the cartilage varies in different places. It is thin towards the free margin and is thicker higher up. The thin tissue consists of vacuolar cartilage cells. When the cartilage higher up becomes thicker, the center of the cartilage often shrinks and becomes necrotic and connective tissue grows into it, replacing the necrotic part. Furthermore, when the cartilage becomes necrotic, the perichondrium at the outside begins to proliferate and to form a plate of new cartilage cells, which later produce much hyaline tissue; then again some solution processes may take place.

The conditions which lead to a necrosis of the transplanted normal cartilage (*i.e.*, lack of foodstuffs and oxygen) have the same effect in the non-transplanted normal cartilage. Furthermore, the perichondrium in this instance also produces plates of new cartilage

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alongside the necrotic cartilage. Connective tissue grows into the necrotic areas under normal conditions, just as in the case of transplantation. There is a structural difference between the xiphoid cartilage of the rat and of the guinea-pig, in the former the tendency to vacuolization on the part of the cartilage cells being greater than in the latter.

Autotransplantation of Cartilage

A complete series of autotransplantations of cartilage was not undertaken in the rat, but pieces were examined between the seventeenth and twenty-sixth day after transplantation. On the whole, the cartilage is found in a normal condition, surrounded by perichondrium and fat tissue. The perichondrium is always free from lymphocytes. The fat tissue is either quite normal or occasionally it shows some necrotic areas. In some cases it is free from infiltration, but in other cases there are very slight collections of lymphocytes around vessels. The presence of the lymphocytes is not due to specific homoio-toxins, but is probably caused by necrotic changes and other accidental alterations in the fat tissue. Some small necrotic areas are also quite commonly found in the cartilage either at the end, or in the central parts of the transplanted tissue, where it is thicker and where there is some scarcity of food or oxygen. In certain cases the necrotic central part of the cartilage becomes dissolved. Buds of connective tissue invade and replace the necrotic areas. Occasionally necrotic pieces of cartilage are surrounded by connective tissue which has grown into the transplant. Furthermore, alongside areas of dead cartilage perichondrium produces, as usual, new plates of perichondrial cartilage. We see then that the autotransplant shows reactions similar to those observed in the normal non-transplanted cartilage.

Homoiotransplantation of Cartilage

We shall discuss successively Series I, II and III of transplantations. In order to summarize our results, we shall adopt the same system of grades which we used in the case of the thyroid, and in each animal we shall compare the grades of the transplant with those of the corresponding thyroid transplant, obtained from the same animals. As we noticed previously in the guinea-pig, so also in the

rat the homoio-reaction is less marked in cartilage than in thyroid transplantations. Thus the differences between different homoio-transplants are less distinct in the case of cartilage and the grading is made somewhat more difficult. Furthermore, when cartilage is used, the transplants are found better preserved than the corresponding transplants of thyroid and the grades of reaction are therefore higher than with the latter tissue. Notwithstanding this difference between thyroid and cartilage, there will be apparent a correspondence between the results obtained in both tissues when transplanted into the same animal. We shall give first a summary of the results obtained in each series and compare in each case the grades of reaction against cartilage and thyroid and then cite a few examples to indicate the changes taking place in the cartilage transplants at various periods.

SERIES I. WHITE RATS, NOT RELATED

A. *From 20 to 25 days. 20 days.* (*a*) Only little of the surrounding fat tissue left. In places marked, in other places moderate, but always a distinct mantle of lymphocytes around cartilage; some penetration of lymphocytes into perichondrium. Around necrotic cartilage regenerated perichondrial cartilage plate. Grade 2 (thyroid grade 1). (*b*) Marked fibrous substitution of fat and areolar tissue; some fat tissue left; marked lymphocytic infiltration around cartilage and in fibrous tissue. Grade 1.5 (thyroid grade 1). (*c*) Cartilage partly living, partly necrotic. Fat tissue around cartilage; slight lymphocytic connective tissue thickening in fat tissue. No lymphocytes. Grade 4 (thyroid grade 4). (*d*) Much necrotic cartilage, regenerated perichondrial cartilage plate; fat tissue with fibrous nodes; few lymphocytes. Grade 3 (thyroid grade 1). (*e*) Necrotic center of thick cartilage invaded by connective tissue. Thickening of perichondrial tissue; new formation of perichondrial cartilage. Considerable areolar tissue left, but much of it invaded and replaced by fibroblasts; very densely infiltrated where fibroblasts proliferate. Grade 2.5 (thyroid grade 3 or 3.5). (*f*) Areolar fat tissue around cartilage, but also some dense fibrous tissue. In fibrous tissue, especially around remnant of fat tissue, here and there lymphocytic infiltration; mantle of lymphocytes around cartilage. Bone and proliferative zone of cartilage adjoining bone, preserved. Osteoblasts and osteoclastic giant cells. Grade 3 (thyroid grade 2).

21 days. Good cartilage. Fat tissue partly invaded and surrounded by connective tissue; much lymphocytic infiltration in connective tissue of fat. Bone partly preserved with osteoblasts. Much lymphocytic infiltration around muscle tissue. Grade 3 (thyroid grade 3).

24 days. Necrotic center of thick cartilage; perichondrial regenerated plate. Connective tissue growing into necrotic cartilage. Moderate connective tissue thickening in fat tissue. Grade 3.5 (thyroid grade 2).

25 days. Cartilage partly necrotic; regenerated perichondrial plate. Medium fibrous thickening of areolar tissue; lymphocytic infiltration around cartilage and fat tissue. Grade 3 (thyroid grade 1).

B. *From 30 to 40 days. 30 days.* (*a*) Good cartilage surrounded by areolar tissue. Some increase in connective tissue. In areolar tissue a number of strands of lymphocytes; also around cartilage some clumps of lymphocytes. Grade 3 or 3.5 (thyroid grade 2.5). (*b*) Necrotic areas are seen in thick cartilage; regenerated perichondrial cartilage plate. Increased connective tissue in areolar tissue and around cartilage. Fairly marked lymphocytic infiltration around cartilage or along perichondrium; no general infiltration. Grade 3 (thyroid grade 1 or 1.5).

40 days. (*a*) Good cartilage; small necrotic areas. Fibrous, fibrillar connective tissue, which encloses fat cells, around cartilage. Marked lymphocytic infiltration, extending also into periphery of living cartilage. Grade 1.5 (thyroid grade 1). (*b*) Good cartilage; mostly moderate lymphocytic infiltration. Grade 3.5 (thyroid grade 1). (*c*) Good cartilage surrounded by lymphocytes, areolar and fat tissue. Very moderate increase of lymphocytes. Grade 5 (thyroid grade 5). (*d*) Good cartilage, areolar tissue and fat tissue. Some necrosis with perichondrial cartilage regenerated. Very slight lymphocytic infiltration. Grade 5 (thyroid grade 5).

C. *Earlier transplants from 5 to 8 days. 5 days.* Cartilage partly living, surrounded by fat tissue; no connective tissue; no lymphocytic reaction. *8 days.* Cartilage partly living; some perichondrial newformation of cartilage. Good fat tissue and some increase of connective tissue around cartilage. Little nodes of connective tissue in fat tissue, no lymphocytes; chains of muscle nuclei; grade 2.5.

D. *From 10 to 12 days. 10 days.* (*a*) Good cartilage surrounded by fat tissue; some necrotic cartilage; slight connective tissue increase in fat tissue and around cartilage. No lymphocytic infiltration; perichondrial plate. Grade 3 or 4 (thyroid grade 2.5). (*b*) Good cartilage with necrotic areas in center; thickened fibrous tissue; areolar tissue with strands of connective tissue; much connective tissue proliferation; lymphocytic infiltration. Grade 2 or 3 (thyroid grade 2 or 3). *12 days.* (*a*) Good cartilage; some necrotic cartilage, perichondrial plate. Much increase in connective tissue around cartilage and fat tissue. Some epithelioid cells. Some fat still well preserved. Much lymphocytic infiltration. Muscle nuclear chains. Grade 2 (thyroid grade 1). (*b*) Slight increase of connective tissue in fat tissue. Some lymphocytes. Grade 3.5 (thyroid grade 3.5). (*c*) Good cartilage, areolar and fat tissue with strands of connective tissue. Much lymphocytic infiltration affecting, almost exclusively, areolar and fat tissue. Grade 2 (thyroid grade 2). (*d*) Lymphocytic reaction around cartilage in areolar tissue. Small areas of lymphocytes and slight increase of connective tissue in areolar tissue. Grade 2 or 2.5 (thyroid grade 3).

E. *From 13 to 17 days. 14 days.* Much necrotic cartilage, with substitution by perichondrial cartilage, surrounded by areolar and fat tissue with connective tissue strands. Moderately diffuse lymphocytic infiltration, more marked in areolar tissue than around cartilage. Grade 3.5 (thyroid grade 4). *15 days.* Good cartilage; where it is thicker, necrotic areas in center and a little perichondrial cartilage. Areolar tissue mainly fibrous; only remnants of areolar tissue; marked lymphocyctic infiltration in connective tissue of areolar tissue and around cartilage. Grade 1 (thyroid grade 1). *16 days.* (*a*) Much necrotic cartilage, with some perichondrial regeneration. Good fat tissue around cartilage; no connective tissue nor lymphocytic infiltration. Some connective tissue near transplanted muscle tissue and lymphocytes near point where cartilage forms bone. Grade 4.5 (thyroid grade 4). (*b*) Good cartilage; no connective tissue increase. Intense lymphocytic infiltration around cartilage and in fat and muscle tissue. Grade 4. *17 days.* Good cartilage. Some necrotic areas and perichondrial plates. In areolar tissue, nodular connective tissue increase and increase of connective tissue especially around vessels. Moderate lymphocytic infiltration. Bone mostly necrotic. Grade 3 (thyroid grade 2).

In this series the bone adjoining the cartilage was transplanted in some cases. We cite a few of the protocols of cartilage and also of bone transplantation.

After 12 days. (*a*) Chi=Ad. No thyroid found; the homoio-reaction in the cartilage transplant is quite pronounced. The cartilage itself is well preserved, except for an area of necrotic cartilage around which perichondrium produces a new cartilage plate. There is some increase in connective tissue in the fat tissue and around the cartilage, and epithelioid cells have formed in the fat tissue. In most places fat cells are separated by newly formed connective tissue, but some fat tissue is still unaltered. In the transplanted muscle there are either single nuclei or chains of nuclei, some of which are very large. Connective tissue separates the muscle fibers. There is much lymphocytic reaction around the muscle tissue. There is also much diffuse, though not very intense, lymphocytic infiltration in fat tissue, and some lymph nodes are filled with lymphocytes. (*b*) Ad = Po. The thyroid transplant has almost the character of an autotransplant, except for beginning lymphocytic infiltration. The cartilage and fat tissue also resemble auto-tissue in structure; but there is, in the fat tissue, in places an increase in fibroblasts with some lymphocytes. In the capillaries many lymphocytes are occasionally found. Cartilage and thyroid transplants correspond thus to each other.

After 16 days. Ad = Micha rat. There is considerable necrosis in the cartilage; especially where the cartilage is thicker, necrotic areas develop and cavity formation results. Around the necrotic cartilage the perichondrium produces new cartilage in places. Fat tissue around cartilage well preserved, and without any connective tissue increase or lymphocytic infiltration. There is also some transplanted muscle tissue seen and in this area connective tissue proliferation has taken place; some lymphocytic infiltration near cartilage. This piece has almost the character of an autotransplant, although the corresponding thyroid transplant shows a mild homoio-character. Toward the adjoining bone a proliferation of the perichondrial cells takes place and the cells enlarge without vacuoles forming; their cytoplasm stains blue with hematoxylin; intercellular substance is produced and bone formation results.

After 17 days. Micha = St. L. Bone is mostly necrotic, but in some parts bone corpuscles are still present.

After 20 days. From Chi to Ada rat. Cartilage is well preserved. There is mostly areolar connective tissue and fat tissue around cartilage, but also some dense fibrous tissue. In the fibrous tissue some lymphocytic infiltration is noticeable, especially around remnants of fat tissue; otherwise only here and there lymphocytic infiltration in the areolar tissue. Lymph vessels are filled with lymphocytes and a mantle of lymphocytes surrounds the cartilage. In the bone, bone cells are preserved and columns of cartilage cells are seen in the zone where cartilage becomes transformed into bone; there are also large capillaries here. Around the bone there are connective tissue and cells resembling osteoblasts and osteoclastic giant cells. In this case the homoio-reaction in the cartilage is less severe than in the thyroid.

After 21 days. From young Chi to old Ada rat. Cartilage is well preserved and partly surrounded by fat tissue in which there is considerable lymphocytic infiltration; some lymph vessels here are filled with lymphocytes. Connective tissue is growing into the fat tissue and the latter is thus gradually more and more invaded by it, the fat cells being surrounded by and included in the connective tissue. There is considerable lymphocytic infiltration in the connective tissue and much also in the transplanted muscle tissue. Within the lymphocytic masses there seem to be some small myoblasts. The bone is partly preserved, with osteoblasts surrounding it; in other places these cells are lacking. Osteoclasts also are found around the bone. In both thyroid and cartilage transplants, we find marked lymphocytic infiltration and in both the connective tissue is increased; there is much lymphocytic infiltration, especially around the muscle tissue.

We find thus, in this series, bone preserved in the homoiotransplants. In the zone of bone formation from cartilage there may be columns of cartilage cells which produce the material from which bone develops.

Conclusions (Series I)

1. In all essential respects the homoio-reaction against cartilage in the rat is similar on the one hand to the homoio-reaction against thyroid in the rat and in the guinea-pig, and on the other hand to the homoio-reaction against cartilage in the guinea-pig, but there are some quantitative differences. (*a*) In the homoio-reaction

against cartilage in the rat, the lymphocytic and connective tissue reaction is less intense than in the homoio-reaction against thyroid in the rat or guinea-pig. On account of this weaker reaction the differences in the intensity of the homoio-reaction against cartilage in the rat are not so distinct as in the case of the thyroid and therefore the grading is not so easily applied. For this reason it is not possible to establish perfect parallelism between the individual grades of homoio-reaction against cartilage and thyroid in the rat; but in a general way a parallelism can be demonstrated. (*b*) If we compare the homoio-reaction against cartilage in the rat with that in the guinea-pig, we find that in general the invasion of fat and areolar tissue and its substitution by connective tissue is less pronounced in the former than in the latter animal. (*c*) The cartilage withstands more successfully than the thyroid the attack of lymphocytes and connective tissue.

2. In those rats in which the grades of homoio-reaction against thyroid varied between 1 and 2, 3 and 5, and 4 and 5, the corresponding average grades in the case of cartilage were 2.5, 3.84 and 4.3. There is thus a general correspondence between the grades of homoio-reaction against cartilage and thyroid. The homoio-differential against the cartilage and the thyroid of a certain individual is essentially the same on the part of the same host, but secondary factors may cause some minor differences.

3. It is possible to some extent to separate, in individual cases, lymphocytic from connective tissue reaction. Lymphocytic reaction may, at a certain time, be noticeable when the connective tissue reaction is weak, and conversely the connective tissue reaction may be marked when the lymphocytic reaction is almost lacking; but in general there is a parallelism between these two kinds of homoio-reactions in the case of rat cartilage as well as of rat thyroid transplantation.

4. The homoio-reaction against cartilage is usually quite distinct on the tenth day after transplantation, but it may begin a few days earlier. It reaches its greatest intensity in the fourth week, but there is a definite indication that at a still later date the intensity decreases again. This fact will come out more clearly in Series II, in which the observation extended over a longer period of time.

SERIES II (1 TO 85 DAYS)

In this series the rats had in some cases been obtained from the same breeder. Fifty-two pieces of cartilage were examined microscopically. They were taken out at intervals varying between one and eighty-five days following transplantation.

A few abstracts from protocols may be cited to illustrate the changes which take place in this series at different periods after transplantation.

14 days. Cartilage transplant, grade 3 (thyroid grade 1). Where cartilage is thin, it is well preserved; where it is thick, there is central necrosis. Some of the thick cartilage is dissolved. Near the thick part, in places perichondrium also necrotic. Where cartilage is necrotic and perichondrium preserved, perichondrium proliferates slightly. At one point perichondrium is detached from thick cartilage and forms a plate of perichondrial cartilage, which runs parallel to transplant; it is still adherent to old cartilage at one end. In the center thick cartilage becomes soft, partly dissolved and forms apparently a myxoid tissue. At last cells and fibrils disappear. The surrounding fat tissue is markedly infiltrated with fibroblastic and fibrillar connective tissue which includes fat cells; considerable area of fat tissue still preserved. Some lymphocytes in this connective tissue, but not large masses. Some lymph vessels fairly well filled with lymphocytes.

16 days. Grade 3.5 (thyroid grade 2.5). Cartilage on the whole well preserved. Where it is thicker, center is necrotic. In some places where cartilage is thick, the perichondrial cartilage also is necrotic. Connective tissue grows in and sequesters necrotic piece. Cartilage surrounded on one side by fibrillar fibrous connective tissue, on the other side by fat tissue. Around cartilage some fibroblasts produce fibrillar tissue. Through solution, cavities develop in fat tissue. Thus cells become free and produce apparently a loose connective tissue. Distinct lymphocytic infiltration in places around cartilage. Around necrotic cartilage there is perichondrial newformation. Proliferating perichondrial cells produce cartilage tissue in necrotic center.

19 days. Grade 3.5 (thyroid grade 2.5). Where cartilage is thin, it is preserved; where it is thicker, it is necrotic. Around the latter places there is slight perichondrial cartilage formation. Some con-

nective tissue grows into necrotic hyaline cartilage and organizes it. Perichondrium, detached from cartilage near necrotic thick end, forms new plate of cartilage. Fat tissue surrounds cartilage, but some thickened connective tissue septa emanate from vessels, and here is also some lymphocytic infiltration.

20 days. Grade 3 (thyroid grade 3). Good cartilage surrounded by areolar tissue. Dense lymphocytic infiltration around cartilage and in areolar tissue. A few lymphocytes penetrate margin of cartilage; newformation of cartilage. Thyroid resembles in this case a lymph gland. Correspondingly there is marked lymphocytic infiltration in cartilage transplant; but absolutely the infiltration is less than in thyroid.

20 days. Grade 5 (thyroid grade 5). Good cartilage; central necrosis with perichondrial plate. Good areolar tissue; no new connective tissue; no lymphocytic infiltration around cartilage.

25 days. Grade 3 (thyroid grade 3). Vacuolar cartilage cells. Perichondrial regeneration. Newformation of connective tissue. Marked lymphocytic infiltration in fat and connective tissue.

30 days. Grade 3 (thyroid grade 2). Much perichondrial regeneration. Some perichondrial cartilage proliferation into necrotic cartilage. Well preserved fat and connective tissue around cartilage. Distinct lymphocytic infiltration in fat and connective tissue, but much less than in thyroid.

40 days. Grade 4.5 (thyroid grade 4.5). Good cartilage; some slight changes observed. The cartilage transplant is more like an autotransplant than is the thyroid. Where there is necrosis, some newformation of perichondrial cartilage has grown into necrotic area. In areolar and fat tissue some lymphocytic strands. Fibroblasts produce, in places, slight newformation of fibrous tissue and thus we find here and there some fibrous thickening.

50 days. Grade 6 (thyroid grade 5). Cartilage shows auto-reaction. No increase in fibrous tissue; no lymphocytic infiltration. In the thyroid transplant we find on the whole an auto-structure, but there are, in various places in the center as well as in the periphery, collections of lymphocytes, especially around blood vessels. They penetrate also slightly between acini. Lymph vessels in center and in periphery are filled with lymphocytes. Again the thyroid is a more sensitive indicator for homoio-toxins than the cartilage.

85 days. Grade 6 (thyroid grade 5.5). In thick cartilage, there

are necrotic areas and solution in center. The necrotic areas reach to connective tissue in places and the latter grows in and replaces necrotic material. At other side of cartilage much perichondrial cartilage regenerated, which may take the place of necrotic cartilage. The newly formed cartilage has at first small vacuoles which gradually enlarge. Even where cartilage is thinner, there are necrotic spots, substituted in part by regenerated perichondrium which forms cartilage. Perichondrium is thickened, and more so around thick than around thin cartilage. There is a large amount of good fat tissue around the transplant; no connective tissue thickening; no lymphocytic infiltration. Cartilage transplant corresponds to an autotransplant.

Conclusions (Series II, 14 to 85 days)

1. In this series again a certain correspondence between the intensity of the homoio-reaction against the thyroid and the cartilage transplants is noticeable. In those animals in which the grades of reaction against the transplanted thyroid vary between 1 and 2, 2.5 and 4, and 5 and 6, the average grades against the cartilage transplant are respectively 3.1, 3.5 and 5.5. Furthermore, it is again apparent that the lymphocytic and connective tissue reactions are more marked in the thyroid than in the cartilage transplant. This difference is especially noticeable in those animals in which there is a marked homoio-reaction; in the animals in which the homoio-reaction is weak or lacking altogether in the case of the thyroid, it is also weak or lacking in the case of the cartilage. The destruction of tissue by lymphocytes and connective tissue is, on the whole, much greater in the thyroid transplant than in the cartilage. Moreover we are apt to find, in cases in which the thyroid transplant is so markedly invaded by lymphocytes that it almost resembles a lymph gland, that the cartilage transplant also is severely infiltrated. If we compare the degree of the homoio-reaction against cartilage in Series I and II, we find it somewhat more marked in Series I in which a relationship between donor and host can be excluded with greater certainty than in Series II. During the period between the nineteenth and twenty-sixth day, we find in Series I an average grade of 2.8, and in Series II an average grade of 3.3. We find again in Series II that the severity of the homoio-reaction gradually decreases and that in the course of time a recovery takes place in the

cartilage transplant. Thus between the nineteenth and twenty-sixth day the average grade is 3.3, while between the thirtieth and eighty-fifth day it is about 5.

2. The necrotic parts of the cartilage, especially those in the center of the transplanted tissue, can be replaced either by new cartilage produced through the activity of the adjoining perichondrium or by connective tissue which invades the necrotic areas. Which of these two processes occurs depends upon the state of preservation and reactivity of the perichondrium. It seems that necrosis of adjoining cartilage acts as a stimulus upon the perichondrium, which induces it to proliferate. In addition the perichondrium may proliferate alongside the necrotic cartilage and form a plate of new cartilage without actually replacing the dead parts. The new plates may be separated from the necrotic cartilage by a layer of connective tissue.

3. In several cases we observed that, through solution of cartilage, cartilage cells become freed from the surrounding intercellular substance and assume the appearance of myxoid connective tissue.

4. The recovery which we observed in the cartilage transplants at about the fortieth and fiftieth day following transplantation, is evident in the decrease in the intensity of the lymphocytic infiltration and in the quiescent state in which we find the connective tissue. Thus in several cases at later stages the transplant resembles autotransplants. Similar results were obtained in subsequent experiments with cartilage transplantation, and while it is possible that accidental variations in genetic relationship between donor and host may have played some part in bringing about the excellent condition in some of the transplants at later periods, there can be little doubt that a gradual diminution in the severity of the reaction against the strange tissue, on the part of the host tissue, does actually take place. An adaptation, perhaps akin to active immunity, may possibly produce this change in reaction.

We still have to consider the changes which take place in the cartilage and surrounding tissue in the first ten days after transplantation.

1. *In the first two days*, we find the cartilage itself well preserved, except where as a result of injury some necrosis occurred. Where the cartilage is thick, the center is necrotic and in the periphery a perichondrial cartilage plate has been produced. This took place

before transplantation. The fat and areolar tissue around the cartilage is on the whole preserved, but from the outside polynuclear leucocytes and some lymphocytes penetrate it. These cells may also enter into the cut end of the cartilage, when the cut has caused some necrosis; they furthermore invade necrotic cartilage elsewhere, as well as necrotic muscle tissue. The polynuclear leucocytes vary in number in different specimens, but on the whole they are not very numerous. They first surround the transplanted fat tissue and from here penetrate toward the center of the transplant.

3 days. Transplanted cartilage and fat tissue are well preserved. A few polynuclear leucocytes are seen here and there; also a few scattered lymphocytes around blood vessels. There is a slight connective tissue proliferation in the periphery around the fat tissue.

4 days. Polynuclear leucocytes have disappeared; lymphocytes are not observed. No connective tissue proliferation noticeable, except where connective tissue grows into necrotic cartilage. The greater part of cartilage preserved, but some central parts are necrotic and here solution processes have taken place. In the periphery, perichondrial cartilage had proliferated and produced new cartilage before the transplantation. Only small parts of the transplanted fat are necrotic.

6 and 7 days. Condition is similar, except that some proliferation of connective tissue is taking place and some lymphocytes and large mononuclear cells are seen around vessels; also in lymph vessels some lymphocytes and a few polynuclear leucocytes are seen. Here we have the first evidence of the homoio-reaction. This homoio-reaction is a little more distinct after seven days; lymphocytes and fibroblasts are seen in the interstices between the fat cells. Some septa in the fat tissue are thickened by lymphocytes and large mononuclear cells.

8 days. The fibroblasts have produced some connective tissue around the cartilage. Here, as well as in the corresponding thyroid transplant, lymphocytes are lacking. In a transplant of Series I at this period we find, at various points in the areolar or fat tissue, newly formed small nodes of connective tissue. In the muscle tissue some chains of nuclei can be observed. No lymphocytic infiltration; some perichondrial cartilage formation.

9 and 10 days. Cartilage mostly preserved surrounded by fat tissue, in which fibroblastic invasion has caused some connective

tissue thickening. In these connective tissue septa, are lymphocytes which penetrate also between fat cells. There may be in places already considerable substitution of fat tissue by connective tissue. Connective tissue with some lymphocytes is also growing into and replacing necrotic parts of the cartilage. Where cartilage is necrotic, there is either connective tissue proliferation or perichondrial cartilage regeneration, with plate formation already described. In various specimens there are variations as to the intensity of the lymphocytic and connective tissue reaction and they correspond to similar variations in the corresponding thyroid transplants.

Conclusion (Series II, 1 to 10 days)

We see then that in the first three days there are slight collections of polynuclear leucocytes migrating into fat tissue in the direction toward the cartilage. They disappear after this period. Between the sixth and eighth day there begins a new formation of connective tissue and a slight infiltration with lymphocytes, which varies in strength in different specimens. There is a correspondence between the changes which take place in cartilage and in thyroid transplants at the same periods of time.

Series III

This series includes transplantation of cartilage, of surrounding tissue, and in some cases of bone from white rats to cream and hooded rats. Simultaneously thyroid was transplanted into these animals.

1. *From white rats to cream rats. 7 days.* The greater part of cartilage necrotic; fat tissue partly necrotic. Connective tissue growing into living fat tissue. Connective tissue capsules around transplant. Bone necrotic. Moderate lymphocytic infiltration.

20 to 21 days. (*a*) Grade 2. Good cartilage; some newly formed fat tissue, with much fibrous tissue; moderate lymphocytic infiltration. Bone and zone of growing cartilage necrotic. (*b*) Grade 1.5. Limited amount of perichondrial cartilage. Much connective tissue; some fat tissue still left; dense lymphocytic infiltration. (*c*) Grade 1.5. Cartilage partly alive, partly necrotic. Around necrotic cartilage areas of newly formed perichondrial cartilage. Much connective tissue around cartilage and in fat tissue. Much lymphocytic infiltration. (*d*) Grade 2. Cartilage largely preserved, but some

necrotic areas. Fat tissue with increased connective tissue; medium lymphocytic infiltration. Bone necrotic, some fibroblasts invading it. Near bone a collection of what appear to be muscle cells, with large chains of nuclei. (*e*) Grade 2. Some bands of perichondrial cartilage. Much fibrous thickening in the fat tissue, but the lymphocytic infiltration here only moderate. Bone and bone-forming area of cartilage necrotic. (*f*) Grade 1.5. Cartilage partly alive, partly necrotic and surrounded by fibrous tissue, which has also replaced necrotic fat tissue. Preserved fat tissue with fibrillar thickening; moderate lymphocytic infiltration in some places, marked lymphocytic infiltration in others.

2. *From white rats to hooded rats.* (*g*) Grade 1.5. Fat and cartilage partly necrotic. Considerable increase in connective tissue; much lymphocytic infiltration. (*h*) Grade 1.5. This cartilage well preserved; where it is thicker, much necrosis in center. In periphery some newformation of cartilage; very rudimentary cartilage plate. Much connective tissue formation and lymphocytic infiltration. Bone and cartilage-growth zone necrotic. (*i*) Grade 1.5. Only parts of cartilage necrotic. Cartilage surrounded by fat tissue in which there is much connective tissue newformation. In places some lymphocytic infiltration in connective tissue around cartilage, but here, on the whole, it is moderate. Around bone, lymphocytic infiltration somewhat more marked. In bone-producing zone of cartilage, nuclei shrunken or chromatolytic and cartilage cells vacuolar. Bone entirely or almost entirely necrotic. (*j*) Grade 1.5. Cartilage vacuolar, partly necrotic, surrounded by fibrous tissue. Much fibrous tissue increase in and around fat tissue. In fibrous tissue around cartilage and in fat tissue, marked lymphocytic infiltration. Some epithelioid and giant cells in fat tissue. (*k*) Grade 1.5. On the whole good cartilage; only where it is thicker, a great part of it shrunken and necrotic. In surrounding tissue much increase of connective tissue, but some fat cells left. Dense lymphocytic infiltration around cartilage and in connective tissue, also in connective tissue around bone. Bone necrotic with some connective tissue growing into it. Cartilage-growth zone near bone is necrotic. (*l*) Grade 1.5. Good cartilage. Where it is thicker, some necrosis in center. Fat tissue partly replaced by fibrous tissue. Some coalesced fat cells. Dense lymphocytic infiltration in fat tissue and connective tissue around cartilage. Lymphocytes penetrating slightly into

perichondrial tissue; between fat cells marked lymphocytic infiltration.

A comparison of the grades in this series with the grades in the first series of transplants, both examined seventeen to twenty-five days after transplantation, is of interest. In the transplants from white to cream rats the average grade is 1.75; from white to hooded rats it is 1.5. The corresponding grades of transplants from white to non-related white rats is as follows: *17 days:* 2.75. *20 days:* 2, 1.5, 4, 3, 2.5, 3. *21 days:* 3. *24 days:* 3.5. *25 days:* 3. The average grade is 2.8. The transplants are therefore decidedly more injured after transplantation into cream and hooded than into white non-related rats. A similar result had been obtained in the thyroid transplants. Here the transplants were completely or almost completely destroyed in the cream and hooded rats, while in Series I the result was somewhat better. Furthermore, in the case of both thyroid and cartilage transplantations, the cream rats were slightly more favorable as hosts than the piebald rats. In this series also the destruction went further in the transplanted thyroid than in the transplanted cartilage.

Conclusions (Series III)

An analysis of the processes which lead to these results shows the following: That as early as seven days after transplantation, there is much fat and cartilage tissue found to be necrotic, and some connective tissue growth into living fat tissue, as well as around the transplant, has taken place. The lymphocytic infiltration is moderate and less intense than in the case of the thyroid. After twenty to twenty-one days, the cartilage may be well preserved, except that there are found central necrosis and solution processes in places where the cartilage is thick. In a number of instances also other parts of the cartilage are found necrotic; but this is probably due to coincidence, because in other cases the cartilage is well preserved. In contradistinction to the cartilage, a complete destruction of the transplanted thyroid has taken place. Even a newformation of perichondrial cartilage can occur around necrotic areas of this tissue, but the regeneration is limited in amount; in some cases it only leads to production of abnormal structure resembling myxoid tissue. There is, therefore, noticeable on the part of the toxins an inhibiting influence on the regeneration of perichondrium. The transplanted

bone in this series is found completely or almost completely necrotic; also the proliferating zone of the cartilage adjoining the bone is invariably necrotic. The nuclei here may be shrunken or chromatolysis may occur and the cartilage cells are vacuolar. The toxic substances produced as a result of incompatability between transplant and host are injurious to the preservation of this tissue.

There is in this series always much newformation of fibrous tissue in the transplant, usually around the cartilage and always in the fat tissue; a great part of the latter may be replaced by fibrous tissue and only some parts of fat tissue are left. In the remaining fat, epithelioid and giant cells are found in some cases; also a coalescence of fat cells is occasionally observed. Connective tissue may grow into the necrotic cartilage, taking its place and penetrating in places into the bone. The lymphocytic infiltration is usually marked, but in some cases more moderate. These cells are found in the fibrous tissue around cartilage and in the fat tissue, around blood vessels and in lymph vessels. There is also noted lymphocytic infiltration around the bone. In one case remnants of muscle tissue with nuclear chains are found. On the whole, therefore, the reaction on the part of connective tissue and lymphocytes against the transplant is very marked. It is more intense than in Series I and II. In addition there is found in Series III complete necrosis of bone and of bone-forming cartilage. The regenerative activity on the part of the perichondrium is not entirely destroyed but decidedly inhibited.

Homoiotransplantation of Bone Marrow

In the first series six transplantations of bone marrow were carried out, pieces being taken out for microscopic examination after 8, 12, 16, 17, 20 and 21 days. We found in every case a growth of connective tissue, usually accompanied by some lymphocytes, into the bone marrow, and thus a partial substitution of bone marrow by fibrillar connective tissue; only in one case was there bone marrow entirely replaced by fibrillar connective tissue.

If we compare these results with those obtained in the third series, we find in the latter that the destruction of bone marrow is more intense. The bone marrow is in every case completely necrotic and supplanted by fibrillar connective tissue, which in a number of cases is accompanied by lymphocytes. Some areas of solution are

also observed in the bone marrow. As early as seven days after transplantation in Series III the bone marrow is completely necrotic, and it does not have the power to recover from the injury received as a result of homoiotransplantation.

Summary

1. Three series of experiments were carried out, in which cartilage was transplanted in three groups of rats which differed from each other in the degree of relationship between donor and host in each series.

A definite correspondence was established between the degree of genetic relationship between donor and host in each series and the severity of the reactions against the transplant on the part of the host tissues, in particular of the lymphocytic infiltration and the invasion and replacement of transplanted fat tissues by fibrous tissue. This applies to the homoio-reactions of Series I and II and also of Series III. In the latter, where we have to deal with a further-going genetic difference between host and transplant, we find in addition a necrosis of bone and of the bone-producing zone of proliferating cartilage cells. Furthermore, while in Series I and II the regenerative function of the perichondrium is not interfered with, it is inhibited, although not entirely prevented, in Series III.

2. The difference between Series I and II on the one hand and Series III on the other hand comes out also in the fat tissue of transplanted bone marrow. In Series III it becomes entirely necrotic and is replaced by fibrous tissue at an early date, while in Series I and II part of it may remain active for some time. In general, bone marrow is a sensitive tissue which, under the influence of homoio-toxins, is readily injured after transplantation and does not at all or only very incompletely recover after homoiotransplantation. It thus seems to behave in a similar manner to unstriated muscle of uterus after transplantation.

3. Under conditions in which thyroid tissue is entirely destroyed by the reaction of the host tissue, cartilage remains alive to a large extent. If in these series lack of close genetic relationship causes any partial necrosis of the cartilage at all, it has this effect only in certain cases; even in Series III the cartilage may remain very well preserved.

4. In Series I and II the homoio-reaction against cartilage, instead of becoming more intense, on the contrary decreases in intensity in the course of time. An adaptation takes place apparently between transplant and host.

5. In autotransplantation cartilage and fat tissue behave in a manner similar to normal non-transplanted tissue. In places where the cartilage tissue is further removed from the sources of oxygen and food material, degeneration occurs in normal as well as in autotransplanted tissue and the perichondrium may regenerate and produce perichondrial cartilage.

ADENOMAS OF THE ISLANDS OF LANGERHANS *

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Benign tumors of the pancreas are rare, particularly adenomas of the islands of Langerhans. I have been able to find only fifteen cases recorded in the literature. Yet in this paper I am able to report four cases, two from the Boston City Hospital. In addition, one already reported [1] was encountered in studying the material at the Boston City Hospital. Ordinarily but little attention is paid to the pancreas at necropsy, and a small adenoma may easily escape notice. In fact, their discovery is usually due to a chance inclusion in the section taken for microscopic examination. Thus the rarity of the lesion is probably only apparent.

It is difficult to lay down criteria for the diagnosis of this condition. Cecil [2] believes that the masses represent hypertrophied islands rather than true tumors. Certain of the cases reported as adenomas very likely should be interpreted as hypertrophic islands. Thus the large island 1.5 mm. in diameter reported by Ssobelew [3] in a diabetic is probably not a tumor. Similarly, Reitmann's case [4] and that of Herxheimer,[5] both associated with diabetes, hardly have the characteristics of a tumor. The case reported by Lang [6] presents a good deal of difficulty in classification, but can best be considered both hyperplasia and adenoma formation.

In determining the diagnosis of the four cases reported here, the following rules have been followed. The morphology and arrangement of the cells must resemble those of the islands. There must be a definite capsule, with compression of the adjacent pancreatic tissue. The mass must measure at least one millimeter in diameter.

That size alone does not count can be seen from the illustrations of Case III (Fig. 2) and of a large island from a non-diabetic pancreas (Fig. 3). The definite capsule and compression of the surrounding pancreatic tissue can be readily seen in the case of the adenoma, but the large island (Fig. 3) differs from a normal island in size alone.

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In the accompanying table the various cases are summarized.

Case No.	Described by	Sex	Age in years	Diabetes	Cause of death	Size of adenoma
I	Warren	♀	53	—	Carcinoma of ovaries	1.7 x 1.7 x 1.4 mm.
II	Warren	♂	63	—	Myocarditis	1.3 x 1.1 mm.
III	Warren	♀	49	—	Pernicious anemia	1.2 x 1.2 mm.
IV	Warren	♀	48	—	Chronic nephritis	9.0 x 6.5 x 6.0 mm.
V	Nicholls [7]	?	?	?	?	3.0 x 2.5 mm.
VI	Helmholz [8]	♂	65	—	Cardio-renal	5.0 x 5.3 mm.
VII	Morse [1]	♀	44	—	Peritonitis	5.0 x 5.0 mm.
VIII	Morse [1]	♂	46	—	Apoplexy	3.0 x 3.0 mm.
IX	Alezais and Peyron [9]	?	?	?	?	3.0 x 3.0 mm. (?)
X	Heiberg [10]	♀	64	+	Diabetic coma	6.0 x 5.0 mm.
XI	Cecil [2]	♂	63	—	Carcinoma of colon	4.0 x 3.5 mm.
XII	Rollett [11]	♀	25	—	Tuberculosis	11.0 x 11.0 mm.
XIII	Lecomte [12]	♀	42	—	Tuberculosis	100.0 x 70.0 x 40.0 mm.
XIV	Koch [13]	♀	22	—	Bronchopneumonia	14.0 x 14.0 mm.
XV	Priesel [14]	♀	63	—	Lobar pneumonia	12.0 x 10.0 x 9.0 mm.
XVI	Priesel [14]	♂	59	—	Bronchopneumonia	10.0 x 9.0 mm.
XVII	Priesel [14]	♂	60	—	Arteriosclerosis	35.0 x 25.0 x 10.0 mm.
XVIII	Schneider [15]	♂	84	—	Nephritis, broncho-pneumonia	15.0 x 10.0 mm.
XIX	Schneider [15]	♀	70	—	Tuberculosis	50.0 x 45.0 x 30.0 mm.
XX	Lang [6]	♀	39	—	Postoperative bronchopneumonia	Up to 5 mm.

Case I. H–26–177. Female, 53 years old. Clinical diagnosis: Inoperable abdominal carcinoma. Anatomic diagnoses: Carcinoma of ovaries, with metastases to peritoneum and liver.

The pancreas is negative in gross. Microscopic examination shows a thin layer of atypical epithelial cells along the anterior surface, a metastasis from the ovarian carcinoma. On one of the slides, of tissue from the body of the pancreas, a peculiar circumscribed mass is present at the edge of one of the lobules, well within the substance of the gland. On examining the block from which this section was cut, the mass was found to measure $1.7 \times 1.7 \times 1.4$ mm.

The mass is definitely encapsulated with fibrous tissue. There are no elastic fibers demonstrable in the capsule. The surrounding pancreatic tissue is definitely compressed by the mass which consists of cells similar in appearance to those of the islands of Langerhans. No ducts are connected with the tumor.

Under low magnification the tumor is seen to be composed of irregular masses and cords of cells, with a fibrous tissue stroma (Fig. 1). Viewed under high magnification, the tumor cells are rather small and poorly demarcated from one another. They tend to a cuboidal or rarely low cylindrical shape. Blood-filled sinusoids or lacunae are frequently in contact with the cell cords. The nuclei are vesicular with several chromatin masses. No mitotic figures are seen. The cytoplasm takes a pinkish stain with eosin-methylene blue. No zymogen granules are present. Owing to the type of fixation (Zenker's fluid with acetic acid) the presence or absence of α- granules could not be determined, but with Bowie's stain, β-granules could be demonstrated.

CASE II. A-22-149 (Peter Bent Brigham Hospital). Male, 63 years old. Clinical diagnosis: Myocarditis with decompensation. Anatomic diagnosis: Chronic vascular myocarditis.

The pancreas is negative in gross. One of the slides for microscopic examination shows a rounded mass 1.3×1.1 mm. with a thin fibrous tissue capsule. The tissue shows moderate postmortem changes. Those acini near the mass are compressed. Islands of Langerhans are numerous in the section, and this, together with the size of the ducts, suggests that the tissue is from the tail of the pancreas.

The tumor mass consists of low cylindrical cells arranged in cords and irregular masses, with a delicate stroma. Many large capillaries are in contact with the epithelial elements. The cells have an acidophilic cytoplasm and rather large vesicular nuclei with a few prominent chromatin masses. No mitotic figures are present.

CASE III. U13-19 (Boston City Hospital). Clinical diagnoses: Pernicious anemia; syphilis. Anatomic diagnoses: (Primary anemia); hypostatic pneumonia.

The pancreas is negative in gross. Microscopic examination reveals a rounded encapsulated mass, 1.2 mm. in diameter, with moderate distortion of the surrounding parenchyma from its pressure. There is a moderate increase in fibrous tissue throughout the pancreas, with slight infiltration by mast cells and lymphocytes. The islands are negative.

The tumor is made up of irregular masses of cells embedded in a fibrous tissue stroma (Fig. 2). These cells closely resemble those of the islands. They are roughly cuboidal with indistinct cell bound-

aries. The cytoplasm is acidophilic, with only a few granules. The nuclei are large, rather clear, with prominent masses of chromatin. No mitotic figures are seen. Capillaries are in intimate contact with the cell masses. There is no suggestion of the presence of ducts.

CASE IV. A-09-101 (Boston City Hospital). Negress, 48 years old. Clinical diagnoses: Chronic nephritis and mitral stenosis. Anatomic diagnoses: Chronic nephritis and mitral stenosis.

On section of the pancreas, a sharply outlined reddish brown mass is noted at the junction of the head and body of the gland. This mass measures 9.0 × 6.5 × 6.0 mm. Under low power it is seen to be made up of cords and masses of cells supported by a rather delicate fibrous stroma. The blood supply is very abundant. The capsule is definite, varying from 200 to 800 microns in thickness. In the thicker portions, compressed pancreatic acini and one or two islands are included within the limits of the capsule. The surrounding pancreatic tissue is distorted and compressed.

Under high power these cells are seen to be very similar to the island cells themselves (Figs. 4 and 5). They are indefinite in outline, but tend to a cuboidal shape. Their cytoplasm is relatively clear and tends to be acidophilic. The nuclei are relatively clear with several large chromatin masses in them. Mitotic figures are absent. With eosin-methylene blue staining there is no evidence of zymogen granules. In places the cells form lacunae which appear to be filled with blood, and they are in intimate contact with capillaries throughout.

DISCUSSION

That these abnormalities of the islands of Langerhans are much less rare than has been supposed is shown by the fact that three have been encountered at a single laboratory in routine microscopic examination of the pancreatic tissue removed at necropsy. If the pancreas were given less perfunctory attention by the average pathologist, this lesion would probably be found much more commonly.

Their occurrence has no relationship to other more prominent pathologic changes. In only one case (that of Heiberg [10]) was diabetes present, and here no connection between the two conditions is apparent. This lack of correlation with other lesions is rather against the interpretation of these tumors as hyperplastic islands, toward which Cecil [2] leans.

Size alone cannot be accepted as a criterion for these tumors. In Figs. 2 and 3 are shown an adenoma (Case III) and a very large island, respectively. The first was found in the pancreas of a female of 49 years dying of pernicious anemia and hypostatic pneumonia, while the second came from a negress of 54 years, who died of erysipelas. These two islands each measure 1.2 mm. in diameter, whereas the average normal island does not exceed 0.2 mm. In cases of diabetes, islands 1.5 mm. or more in diameter have been occasionally found.

In the two islands considered here, diabetes is not a possible cause of enlargement, nor is any other cause apparent. Fig. 3 is obviously that of an island normal except for its size. The arrangement and character of the cells and their relation to the surrounding tissue all suggest merely an overgrown island. But Fig. 2 (Case III) shows a different type of growth. Here the cells and their arrangement again suggest island tissue, but in addition there is a definite capsule and the surrounding tissue has obviously been compressed and distorted by the growth of the mass.

There is no characteristic age at which these tumors have been found, though most have been discovered in persons dying in the later years of life. These tumors are all slowly growing structures, and probably have existed for years before their presence happens to be detected through postmortem examination. They probably never give rise to trouble during life and have no clinical significance. It is rather interesting that tumors of the island cells, though rare, are nearly always benign, while tumors suggesting origin in the acinar cells in most cases are malignant. This variation would suggest a higher degree of differentiation in the island than in the acinar cell.

Summary

1. Four cases of adenomas of the islands of Langerhans are presented, bringing the number of reported cases up to twenty.

2. These tumors are characterized by resemblance to the islands in arrangement of their cells and in appearance of the individual cell, by absence of mitotic figures, by the presence of a definite capsule and by compression of the adjacent tissue.

3. They are not so rare as the small number of reported cases would lead one to believe.

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DESCRIPTION OF PLATES

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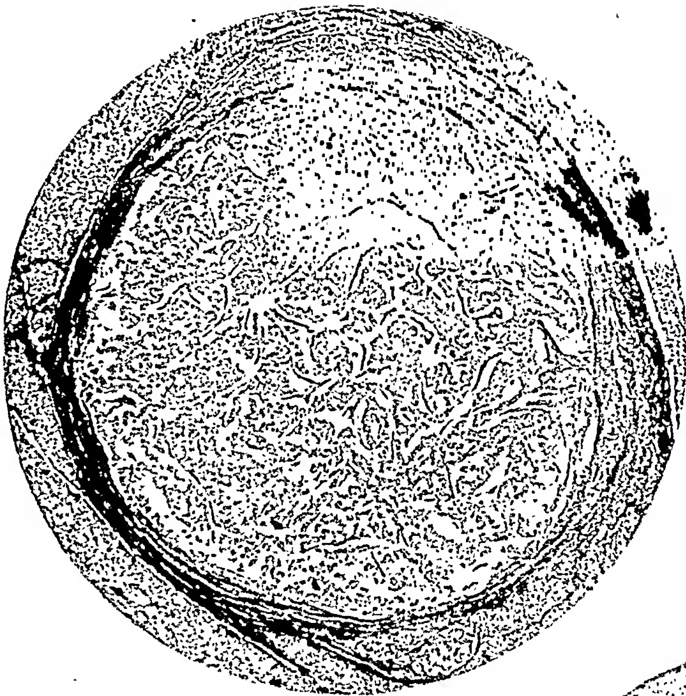
Fig. 1. Adenoma of island of Langerhans (Case I). x 40.
Fig. 2. Adenoma of island of Langerhans (Case III). x 40.
Fig. 3. Large island from normal pancreas. x 40.
Fig. 4. Adenoma of island of Langerhans (Case IV). x 400.
Fig. 5. Normal island from pancreas (Case IV). x 400.



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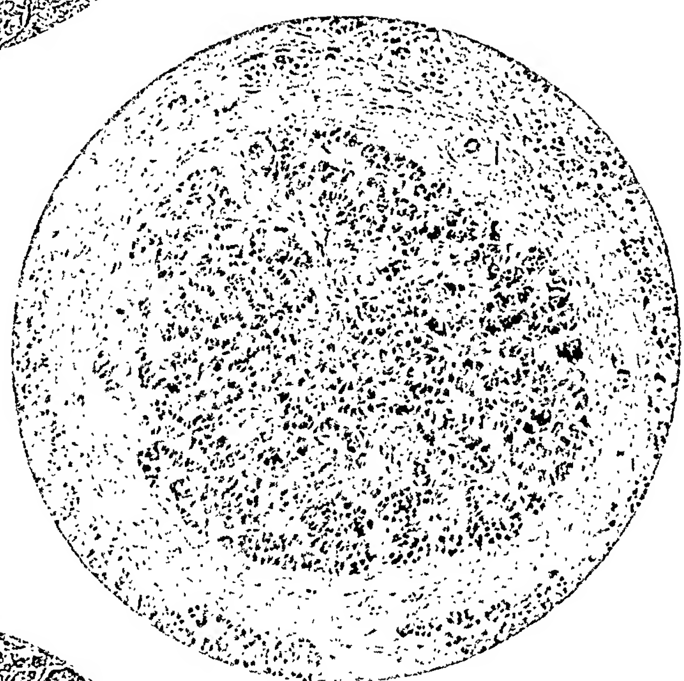

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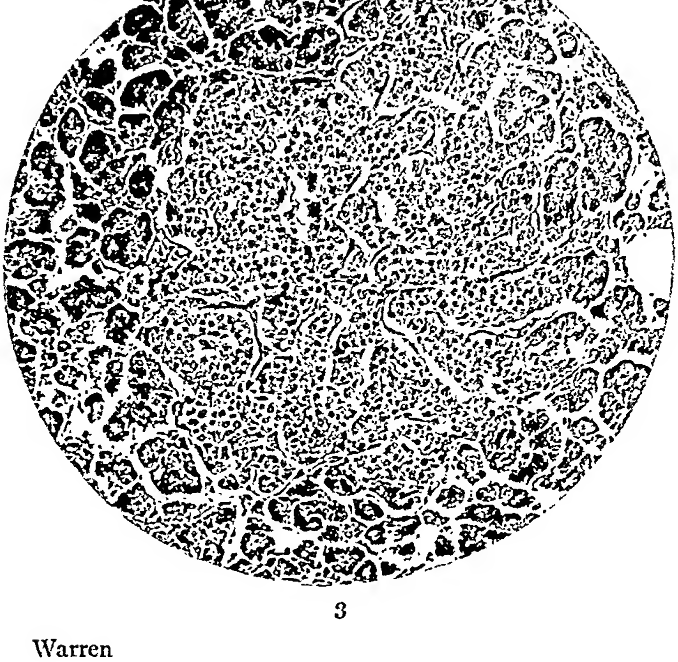

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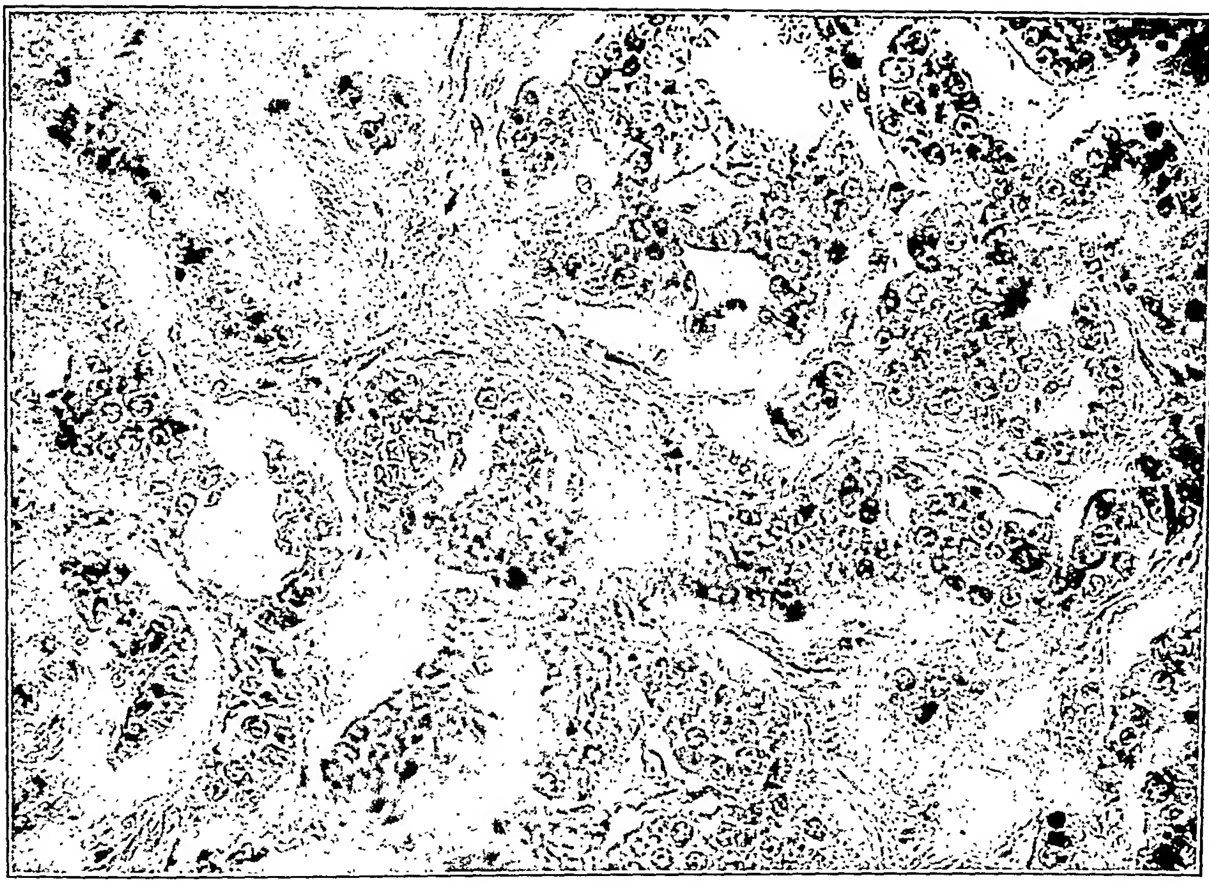
Warren

Adenomas of Islands of Langerhans



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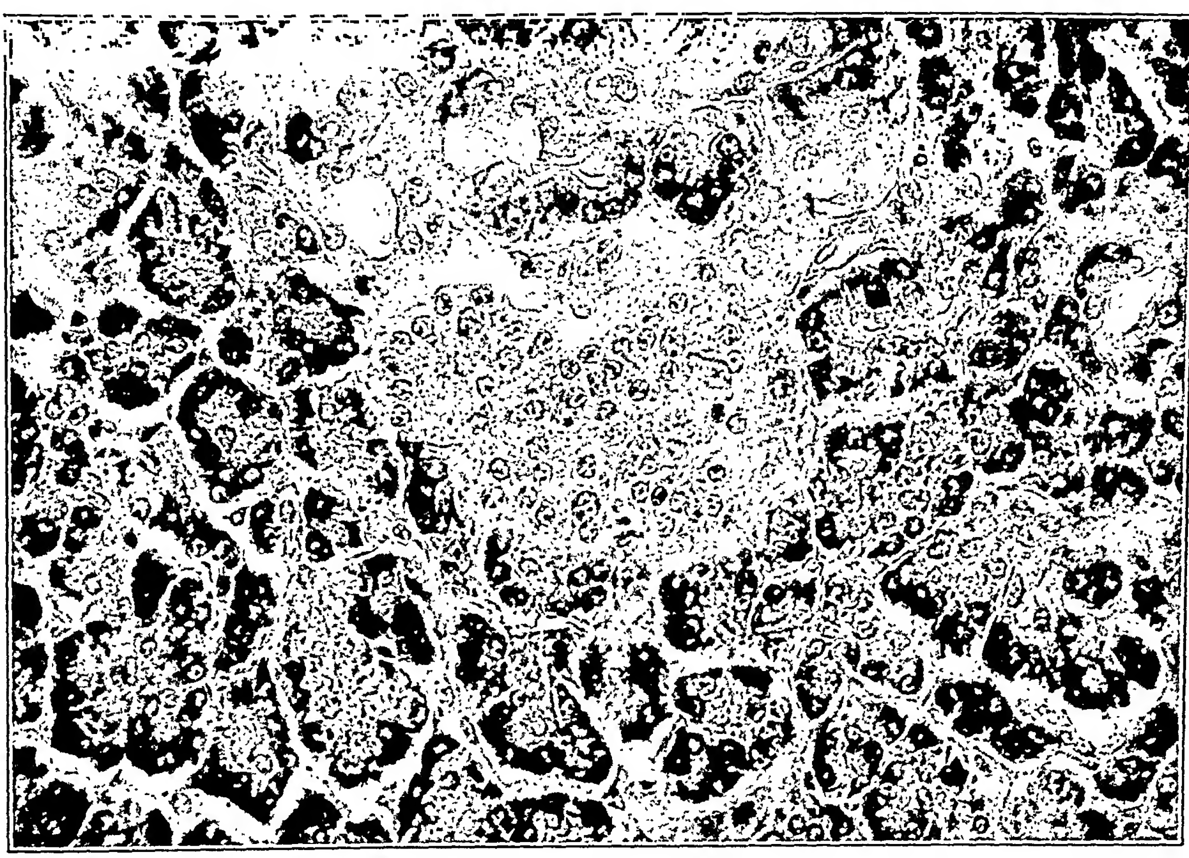

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THE VASCULAR MECHANISM OF THE SPLEEN *

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The study of the pathologic processes in the spleen has been handicapped by an inadequate understanding of the histologic structure of this organ. A perusal of textbooks on histology aids very little. A review of the literature shows that much has been done and many points concerning its vascular system elucidated; but there are many details of structure which are not clear. The question of an open or closed circulation appears to be still unsettled.

This study was undertaken with the hope of obtaining more minute detail of structure and a better understanding of the blood flow through the spleen. In view of the complexity of the subject it is impossible to cover all its phases in one short paper. We are therefore confining our study more particularly to the distribution of the arteries, their relation to the pulp and the nature of the blood flow through this organ.

REVIEW OF LITERATURE

The spleen is a highly vascular organ through which passes a fairly constant flow of blood. This Mall [1] found in the dog to be approximately 5 cc. per minute. Its chief function apparently has to do with purification of the blood and the conversion of hemoglobin. Because of its peculiar anatomy, greatest attention has been paid to its vascular system and the relation of this to the parenchyma. It would appear, however, that the subordinate structures, such as capsule, trabeculae and stroma, are not entirely inert tissues, but by virtue of their muscle and elastic fibers, play an important rôle in

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assisting or impeding the flow of blood through this organ. The peculiar arrangement of the vascular system in its relation to the parenchyma as well as the supporting tissue necessitates, for a proper understanding of the vascular mechanism of the spleen, a study of each portion in detail.

Following death or upon extirpation, the spleen immediately contracts. Barcroft [2] has shown in the cat that following death from drowning or hemorrhage, it may shrink as much as one-half to one-sixth of its original size. For this reason, most experimenters have found it necessary to distend the pulp of the spleen by injections of fixing fluid through the artery or vein in order to demonstrate its histologic features.

Mall,[3] by a study of microscopic sections and macerated specimens of dog spleens, found it to have a capsule made up of connective tissue with muscle and elastic fibers. From the capsule he found prolongations extending into the splenic pulp, forming trabeculae and roughly dividing it into lobules of approximately 1 mm. in diameter. Prolongations from these consisting of muscle fibers alone, divided the lobule still further into smaller compartments. The larger interlobular trabeculae he found intimately associated with veins and so arranged as to pull them open when the trabeculae contract and compress the pulp.

Many workers have described the structure of the splenic pulp. It has been generally recognized as a cavernous, venous network, different in structure from that of any other organ. Leydig (quoted by Billroth),[4] Ludwig Stieda,[5] and others described the cavernous nature of the pulp with its network of star-like cells. Schweigger-Seidel,[6] on the other hand, denied the existence of such a vascular network, claiming that the pulp consisted only of a connective tissue framework supporting the vascular channels and filled with lymph. Oppel,[7] using Golgi's method, demonstrated a network of fibrils in the pulp, which Mall [3] believed were the reticulum fibers. These fibers Mall [8] believed were the supporting framework for the vascular system.

The peculiar structure of the splenic vein wall was first described by Malpighii [9] who noted the cribriform arrangement of their lining cells bound together by fibrils leaving slit-like stomas in their wall. Mall [3] showed that it was only the intralobular veins that had this peculiar type of wall. The large veins embedded in the trabeculae

he found had closed walls. The intralobular veins which were of a cribriform structure, he believed formed a closed system of intercommunicating channels; the veins were widely separated from the arteries of the same order. The fibrils binding the lining cells of the veins together, were believed by most experimenters to be reticulum. V. Ebner[10] thought they were elastic fibrils. Weidenreich[11] later showed they were protoplasmic processes of the pulp cells.

The distribution of the arteries in the spleen was early described by Malpighii[9] who found that the larger branches were clothed at irregular intervals with a lymphoid sheath. Kyber[12] by arterial injection showed that the branches penetrated the lymphoid sheath to break up into a number of non-anastamosing, finer branches in the pulp. What was thought to be the termination of these slender arterial twigs was described by Schweigger-Seidel[6] as "pear-shaped, globoid masses of cells enveloping the arteriole." He called them *Kapillarhülsen*. The term ellipsoid, however, has come to be more generally used to indicate these bodies. Billroth[13] noticed them first in the spleen of the bird, but failed to interpret their function and subsequently failed to attach any importance to them. Schweigger-Seidel studied them in the spleens of sheep, dog, cat and pig. After much diligent search he thought he found one in the human spleen. While in some cases he thought the arterioles ended blindly in the ellipsoid, in other cases he found that they apparently passed through. He thought that there were interstices in the ellipsoids other than the central capillaries, and believed that they were of the nature of a filter apparatus allowing only fluids to pass through. He did not believe that all the arterioles ended in this manner. Some, he thought, ended in other ways and besides there appeared to be too few of them to accommodate all the blood which passed through the spleen. Later Golz,[14] a co-worker of Thoma, using a granular injection mass, showed that the arterioles passed through the ellipsoids and ended in ampullatous dilatations of the vessels. These findings were confirmed by Mall[8] who designated the dilatation of the capillary as "the ampulla of Thoma." Thoma[15] believed that a direct communication was established between the ampullae and the neighboring veins by small communicating vessels, *Zwischenstück*, thus establishing a closed circulation through the spleen. Weidenreich[11] also believed that the arterioles passed through the ellipsoids and in some cases united directly with the

venous channels. In most cases, however, they appeared to break up and fuse with the pulp cells. Mall [3] believed that the ellipsoids were merely an extension of the lymphoid sheath of the Malpighian corpuscles along the arteriole branches. The ampulla of Thoma he divided into three parts: the first third had a distinct wall composed of spindle-shaped endothelial cells; the second third, the ampulla proper, was the dilated portion and had an irregular wall with openings into the pulp; the last third formed a communication with the veins. He was uncertain concerning the nature of this portion. In fact, he was not able to trace a continuous cell boundary from it to the veins, nor was he able to inject this portion of the ampulla from the venous side. Later he [1] came to the conclusion that the ampullae were merely large openings within the spongy pulp spaces — exaggerated pulp spaces.

The nature of the blood flow through the spleen has been the subject of much experimental discussion and study. Poelman [16] discovered that while colored fluid injected into the artery returned by the vein, the reverse was not true. It was impossible, he found, to pass fluid from the venous to the arteriole side. He thought that the arteries terminated in two ways, first by anastamotic arches, and second by an abrupt termination in labyrinthiform cavities. These latter he considered to be merely dilatations of the splenic vein. Poelman therefore must have looked upon the circulation through the spleen as a closed one. Billroth [13] believed that the arteries ended abruptly in the spleen and that the red blood cells after extravasation into the pulp spaces were pressed into the venous sinuses. However, he believed that in some cases the flow was direct, through a closed system as in other parts of the body. Bannwarth [17] believed the ellipsoids and end capillaries opened directly into the pulp spaces. Weidenreich,[11] on the other hand, concluded that the circulation was both by way of the pulp sinuses and by directly communicating end capillaries to the venous sinuses. Janosik,[18] however, believed that the circulation was closed. Thoma,[15] who held similar views, maintained that the difference of opinion was based largely upon the difference in the methods of experiment and in the character of the injection materials used. He said that all who used non-soluble pigment in granular form had obtained results similar to his, and demonstrated a closed circulation. He admitted that the ampullae were permeable to plasma, thus explaining why all workers

using soluble pigment injection masses, got results indicating an open circulation. He believed that while the plasma may percolate through the ampulla into the pulp and thus to the veins, the cellular constituents of the blood were retained within closed channels. Mall [8] at first accepted Thoma's conception of the vascular construction of the spleen and also his view of the blood flow through it. Later, however, after further study and experimentation, he [1] changed his opinion both as to the blood flow and also to some degree as to the vascular structure. He became convinced that the last third of the ampulla of Thoma was cut up by bridges of pulp cells and that the flow of blood was therefore from ampulla to pulp and thence to the vein. In other words he believed that there was an open circulation. In substantiation of this claim, he outlined four experiments which he considered convincing evidence.

Experimental Work

Since it would appear that the pulp is the important functional tissue of the spleen, we will not enter into a detailed description of this phase of the subject at the present time, but will reserve it for a further report at a future date. In our present study we are concerned rather with the distribution of the arterioles, the nature of their termination, their relation to the pulp and venous sinuses, and the nature of the blood flow through the spleen.

TECHNIC

To mark the course and outline of the arteries and their terminal branches a variety of injection material was used, such as celloidin mixtures (thick and thin), oil, hot lard, asphaltum in turpentine, pigment and gelatine solutions. Of these the most satisfactory was gelatine with or without pigment added. Very good injections of the arterial system were made with warm 25 per cent aqueous solutions of plain gelatine. In cases in which it was desired to know the fate of an intravascular injection of particulate matter, a 5 per cent solution of carmine powder in 15 per cent gelatine solution was used. The gelatine was added as a medium to hold the pigment *in situ* when the pulp was distended with a fixative.

Thoma insisted upon the use of a granular pigment in his injection fluid, not only to indicate the course taken by the red blood cells, but also to outline the arteriole channels. We felt, however, that there

was a distinct objection to its use as a substitute for red blood cells, as it was a particulate matter decidedly foreign to the spleen and readily removed by mechanical filtration. In view of the natural fluidity of the red blood cells as shown by Krogh,[19] warm gelatine seemed a much better substitute.

Sheep spleens were used at first, as any number of them could be procured fresh from the abattoir. These had to be removed very carefully from the body in order that the pulp may not be torn by pulling on the vessels. Later, dog, human, cat and chicken spleens were also used. Because of the prominence of their ellipsoids, the dog spleens were found to be the most satisfactory. The arteries were injected with varying quantities of gelatine solution. The quantity of solution used was governed by the size of the spleen and the extent to which the artery and the surrounding pulp was to be injected. It was our desire as a minimum to fill just the arterial system and from this, quantitatively to grade up the injection until the pulp and veins were completely filled, and from these specimens to follow the sequence of events. In some instances, a single intravenous injection of India ink was used, in others repeated injections of pyrrol blue.

The pressure required for injection of the arterial system varied somewhat depending upon the freshness of the spleen and the nature of the injection mass and its temperature and consistence. In the case of warm gelatine solution, if the spleen was injected immediately following death prior to the clotting of the blood, approximately 100 to 200 mm. of mercury were usually found sufficient. On completion of the injection the artery was tied and the organ immersed in cold water to hasten the setting of the gelatine. After allowing sufficient time for this to take place, the spleen was distended to capacity by injecting through the vein either Zenker's fluid with formalin or 10 per cent formalin solution alone. Blocks were taken from various parts of the injected spleen and embedded in paraffin and cut at from 10 to 20 microns. A variety of stains was used, such as Mallory's eosin and methylene blue, phosphotungstic acid-hematoxylin, analine blue, hematoxylin and eosin and Van Gieson's stain. For general purposes, the Van Gieson's stain was found to be the most satisfactory. If the gelatine-injected sections were stained well with iron hematoxylin and carefully differentiated in dilute liquor ferri chloridi, the gelatine would retain the hematoxylin pro-

viding the sections were counterstained for only a few seconds with picro-acid fuchsin mixture. On the other hand, the gelatine injection mass could be stained with the acid fuchsin by differentiating well and staining deeply with the picro-acid fuchsin mixture. The gelatine thus stained outlined clearly the course of the blood through the arteries and pulp sinuses. When carmine pigment was added to the injection mass, picric acid alone was used as a counterstain and the sections were well differentiated. Reticulum stain and elastic tissue stain were also used and silver nitrate injections tried.

RESULTS

We were able to confirm Mall as to the lobular structure of the spleen, divided as it is by trabeculae, consisting of muscle, elastic and connective tissue; also that the interlobular veins are closely associated with the trabeculae, while the arteries occupy a more central position in the lobule.

Pulp. By using a Zeiss binocular microscope with stereoscopic attachments to the oculars, we obtained a three dimension view of our section. We were thus able to see the pulp in perspective and to obtain an accurate idea of its structure. It is found to consist of a vast delicate network of star-like cells having long irregular protoplasmic processes running in all directions uniting one cell with the other and forming attachments to the supporting trabecular framework. Their nuclei are oval, well stained and reticulated. There is very little cytoplasm about the nucleus. The bulk of it extends out in a fine web-like manner and ends in long filamentous processes which fuse with those from other pulp cells. These same cells form a covering for the trabeculae and larger blood vessels. By intravital injections the pulp cells are found to be phagocytic for colloids and particulate matter and therefore reticulo-endothelial in type. With a reticulum stain we were not able to demonstrate a supporting framework for these cells. What appeared to be reticulum is merely the protoplasmic processes of the pulp cells. The pulp cells, thus loosely held together, form with their protoplasmic processes a sponge-like structure having variegated, tortuous, intercellular spaces communicating freely with each other. These pulp spaces vary in size from 0.005 to 0.02 mm. in diameter.

Arteries. The general course of the arteries is found as described by Mall,[8] showing branches penetrating the lymphoid sheath or

Malpighian corpuscle and spreading through the central portion of the lobule. Finer branches consisting of a flattened layer of endothelial cells and surrounded by a muscular layer are found uniformly distributed throughout the pulp and supported directly by the pulp cells. Compared with the veins, they are very much smaller and stouter. After running a course of 0.2 to 1 mm. they appear to end abruptly in globoid masses of cells. These we recognized as the ellipsoids of Schweigger-Seidel. With thicker sections the arterial branches are found to continue through the ellipsoids as a single layer of endothelial cells devoid of a muscle coat.*

Ellipsoids. The ellipsoids in the dog and cat spleens vary in length from 0.17 to 0.24 mm. They are pear-shaped and have a diameter varying from 0.08 to 0.034 mm. In the sheep and human spleens they are much smaller. They are uniformly distributed throughout the pulp, occupying fairly central positions in the "histologic unit," as described by Mall.[8] The arterioles enter at the blunt end, the exit being at the opposite or pointed end. In cases in which the capillary divides within the ellipsoid there is an extension of the ellipsoid cells along the continuing capillaries producing a bicornate or tricornate structure. The cells making up the ellipsoid are found rather closely packed about the capillary which, however, remains quite patent. Using a Zeiss binocular microscope with a stereoscopic attachment for the oculars, we were able to view the ellipsoids in three dimensions and to observe quite clearly their minute detail and structure. With the monocular microscope, one gets the impression that the ellipsoids are a compact mass of cells. With stereoscopic vision we find quite the contrary. The cells are apparently of the same type as the pulp cells, but have more cytoplasm about the nucleus and shorter protoplasmic processes. They are united one with the other by these short processes and are distributed evenly throughout the ellipsoid. We were able to identify intercellular spaces much the same as are seen in the pulp except that they are much smaller, varying in size from one-half to twice the diameter of a red blood cell. Along the margin of the ellipsoid these intercellular spaces communicate freely with the adjacent pulp

* At the recent meeting of The American Association of Pathologists and Bacteriologists, held at Albany, N. Y., April 1, 1926, in my discussion of this subject, an erroneous impression may have been given, namely, that the arterial capillary within the ellipsoid ends blindly.

spaces. The ellipsoid capillary is suspended in the center by the protoplasmic processes of the ellipsoid cells. With the intravital stain the ellipsoid cells are found to be phagocytic for colloid and particulate matter. They are, therefore, functionally of the same type as the pulp cells.

End Arteries. The arteriole enters the blunt end of the ellipsoid and, devoid of its muscle layer, is found to pass through the center as a vessel of capillary size composed of a single layer of endothelial cells. In many cases the capillary divides within the ellipsoid before proceeding into the pulp. The ellipsoid capillaries continue into the pulp as an end capillary for a variable distance up to approximately 0.1 mm. With the binocular microscope, we were able to identify small rounded stomas in the walls of these end capillaries. Toward the termination of the capillaries, their walls in some cases gradually expand in a funnel-shaped manner to blend quite intimately with the pulp. The integrity of the capillary is then soon lost in the cavernous network of pulp cells. In most cases, however, the end capillary terminates in an ampullatous dilatation. These we recognized as the "ampullae of Thoma." They are cone-shaped and have a rounded base. There are large openings in their walls communicating freely with the surrounding pulp spaces. In their first part they appear to have a definite wall made up of the continuing cells of the end capillaries. However, as the vessel expands to form the ampulla, the lining cells fuse quite intimately with the pulp cells and become a part of the pulp tissue. The wall of the rest of the ampulla therefore does not have a definite layer of lining cells, but consists of pulp cells. The ampulla is merely an exaggerated pulp space. In support of this view, we were able in many instances to find the end capillaries expanding and fusing with the pulp cells in a most diffuse and intimate manner without the formation of any cavitation of the pulp tissue. In no case were we able to identify a communicating vessel between the ampulla and vein.

Veins. Coursing through the pulp and supported directly by the pulp cells, are found the large venous sinuses described by Mall and others. They are chiefly in the peripheral portions of the lobule, intimately associated with and in many cases surrounded by the trabeculae. Their walls are well formed. The finer venous branches course freely throughout the whole lobule anastamosing one with the other to form a branching network of collecting channels intimately

associated with the pulp. Their association with the ellipsoids in many cases is quite intimate. At times they almost completely surround them. Their walls, in contradistinction to the larger veins, are incomplete in structure. They are made up of parallel rows of elongated endothelial cells loosely bound together by the protoplasmic processes of the neighboring pulp cells. Slit-like stomas are left between the cells, giving it the appearance of a barrel with alternate staves removed. We were unable to demonstrate any other covering to these vessels. The nuclei of the lining cells are oval and bulge into the lumen. With intravital stains, they are found to be non-phagocytic.

Blood Flow. To demonstrate the blood flow through the spleen, varying quantities of warm gelatine solution with or without carmine pigment were injected into the arteries and veins. Sections were then examined. When a large amount of gelatine is injected into the artery it can be traced through the whole arterial system. The arteries and their branches up to the ellipsoids are found to be impermeable to the gelatine solution. However, it filters through the ellipsoid and appears in fine threads running out in all directions into the surrounding pulp spaces. Quite often the gelatine can be traced directly from the ellipsoid and through the wall of an adjacent venous sinus into its lumen. At other times the venous system is reached only after a devious course through the pulp tissue. Some of the gelatine can be traced on into the end capillaries and ampullae. Here again the permeability of these is manifested. Gelatine threads can be traced through the walls of the end capillaries and ampullae into the surrounding pulp spaces. When minimal amounts are injected and the arterial system alone filled, the arteries and their branches up to the ellipsoid again are impermeable to the injection mass. Beyond this their permeability varies. With minimal injection most of the gelatine passes on through the end capillaries into the ampullae. Here it scatters in all directions in the pulp spaces in an arborescent manner. In places the gelatine oozes through the walls of some of the end capillaries before they expand to form the ampullae.

The arterial terminations are less permeable for carmine pigment; some of it is filtered out by the ellipsoid and here appears in compact masses in the ellipsoid capillaries. Where an intravital injection of

India ink is made, the same filtrative phenomenon is manifested by the ellipsoid. Most of the carbon particles are held in the ellipsoid itself. Some of the granules, however, pass through into the adjacent pulp.

The injection mass, once having reached the pulp, appears no longer to be confined to channels. It is found to have spread out in all directions in a centrifugal manner as fine, filamentous threads. There is no general directional tendency nor any evidence of impediment to flow manifested by undue accumulation of the mass at any one point. Where the injection mass is sufficient, it can be traced by various routes from arterial to venous channels. Where small amounts are used, the bulk of the gelatine passes through the ellipsoid into the end capillaries and the ampullae and from here scatters out into the pulp spaces. In some cases, threads of gelatine can be traced through the tortuous pulp spaces into neighboring venous sinuses.

When the gelatine solution is injected into the venous system, we find that on reaching the intralobular veins it appears to have flowed out in a massive manner into the surrounding pulp spaces. These spaces adjoining the veins are completely filled with the gelatine solution. Very little pressure is required for these injections.

Discussion

After making a careful study of various animal spleens whose vascular system had been injected with gelatine and pigment solutions and the pulp distended to capacity with fixative, we believe that we have been able to demonstrate some of the minute histologic structures of this organ. While its lymphoid elements are important, the essential tissues are the pulp and ellipsoids. The pulp cells by their long, protoplasmic processes, are woven into a lace-like net to form a vast cavernous system of intercellular spaces freely communicating with one another. The cells are thus exposed on all surfaces to the content of the pulp spaces. The pulp and ellipsoid cells, being phagocytic for colloid and particulate matter, are endothelial in function. They are active phagocytic clasmatocytes which Aschoff [20] called reticulo-endothelial cells.

The capsule and trabeculae, besides acting as a supporting frame-

work, in a very large measure control the flow of blood through the pulp spaces. Mall,[1] Tait [21] and others have shown that with the relaxation of the musculo-elastic framework (the capsule and trabeculae), the pulp spaces fill with blood. On contraction the spaces are readily emptied.

The arterial system of the spleen is of unique design. It is non-anastamotic and ends in the cavernous system of pulp spaces. Near the endings of the finer arterial branches the muscle coat of the vessel is replaced by a compact mass of cells, the ellipsoid. These are endothelial in type and function. A stereoscopic view of the ellipsoids shows that they are not only made up of cells of the same type, but also have the same arrangement as those of the pulp. Intercellular spaces can be seen, similar to those of the pulp, freely communicating one with the other and with the contiguous pulp spaces. The ellipsoid spaces, of course, are much smaller. One might therefore look upon the ellipsoids as merely condensed masses of pulp tissue. Hoyer's [22] conception of them as a protective mechanism to the arteriole against undue stress during pressure increases in the venous circulation or against bursting during high arterial pressure, we think is worthy of consideration.

The ellipsoids do not represent the end of the arterial system. The terminal capillaries pass through them into the looser pulp tissue beyond. Here the capillary opens out and fuses with the pulp cells. The ellipsoids while permeable to gelatine solutions are much less so for particulate matter such as carmine pigment. Because of their small intercellular spaces the ellipsoids may serve to filter out very fine materials from the blood which otherwise might pass through the larger spaces of the pulp.

The end capillary or portion beyond the ellipsoid was demonstrated to be permeable to gelatine and with stereoscopic vision we were able to confirm this by discovering small rounded stomas in their walls. Where the end capillaries fused with the pulp, it appeared in most cases to end in an ampullatous dilatation of the vessel. A stereoscopic view of their walls shows that the outer half of the ampulla is composed of pulp cells alone and is incomplete in structure. In many cases the end capillaries fused with the pulp more intimately and no ampulla was discernible. For these reasons we believe that the "ampulla of Thoma" is merely an exaggerated pulp space produced, no doubt, by the concentration of blood flow at this

point. We were unable to find communicating vessels between the ampulla and the vein. From a study of our gelatine-injected specimens, we believe that the end capillaries serve to scatter the blood stream more widely into the pulp system.

The veins are of such structure, size and distribution as to drain readily the whole cavernous system of the pulp in a minimum of time. The walls of the finer branches are incomplete, having slit-like stomas communicating freely with the pulp spaces in a most intimate manner.

By the foregoing means we have determined the nature of the blood flow through the spleen. We believe that the circulation through this organ is an open one. The terminations of the arterial system are such as to scatter the blood diffusely into the pulp. The veins, by virtue of their size and incomplete walls, allow for its rapid emptying. The amount of blood maintained within the pulp is largely controlled by the muscular system of the capsule and spleen.

Conclusion

The fibro-musculo-elastic capsule and trabeculae serve as a skeletal framework for the splenic pulp which it divides into lobules.

The pulp consists of a network of reticulo-endothelial cells whose protoplasmic processes unite with one another to form a labyrinthiform system of intercellular spaces through which the blood flows.

The arterial circulation opens into the pulp by the flaring out and fusing of the end capillaries with pulp cells.

The "ampullae of Thoma" are exaggerated pulp spaces.

The ellipsoids have the same structure and cellular content as the pulp except that they are more compact.

The intralobular veins have incomplete walls, having slit-like stomas which communicate freely with the pulp spaces.

The ellipsoids and end capillaries are permeable to gelatine solution.

Gelatine solutions injected into the artery pass through the pulp before reaching the veins, except where a venous channel lies contiguous with the ellipsoid.

In this latter case, the flow is through the interstices of the ellipsoid and stomas of the vein wall into the lumen of the vein.

There are no direct communicating closed channels between arteries and veins other than the pulp spaces.

The circulation of the spleen is an open one.

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DESCRIPTION OF PLATES

Plate 68

Fig. 1. Ellipsoids with end capillaries. The end capillaries in the upper and lower right hand corners terminate in an ampulla. The two in the upper left hand corner fuse with the pulp cells without the formation of an ampulla. Cross-sections of nine veins can be seen in this photograph.

Fig. 2. Two end capillaries showing their lining cells fusing with those of the pulp without the formation of ampullae.

Fig. 3. Gelatine oozing from the end capillary into the surrounding pulp spaces.

PLATE 69

FIG. 4. Intralobular vein supported directly by the pulp cells. This photograph illustrates the structure of the vein and also the character of the pulp cells.

FIG. 5. Gelatine oozing directly from ellipsoid into adjacent vein.

PLATE 70

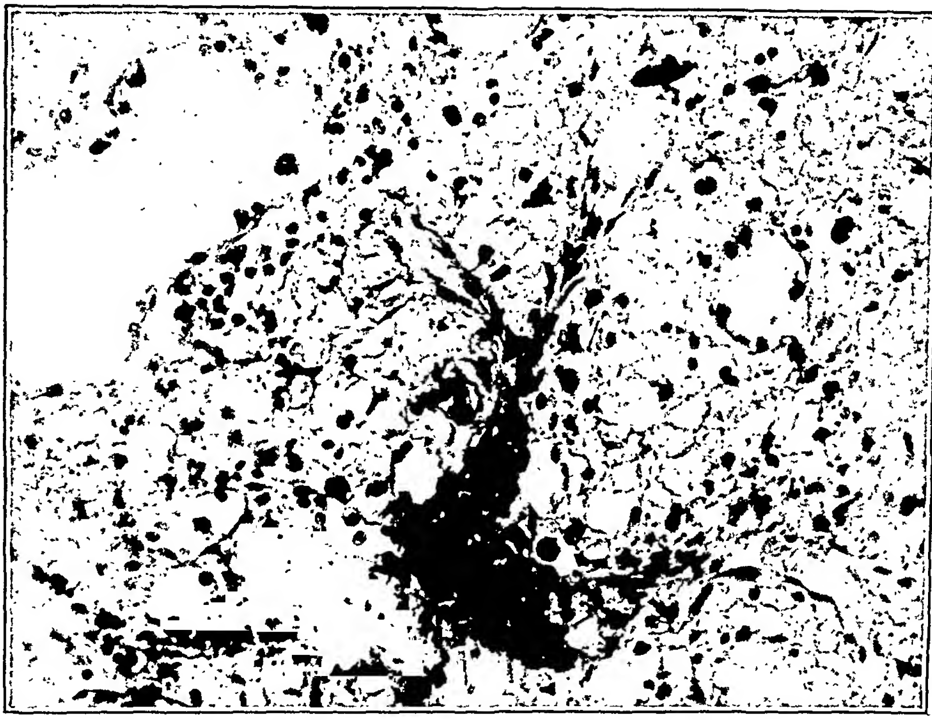
FIG. 6. Dog spleen showing pulp cells covering trabeculae.

FIG. 7. Human spleen. Branching ellipsoids.

FIG. 8. Sheep spleen. Gelatine oozing from vein into pulp.

FIG. 9. Sheep spleen. Gelatine oozing from ellipsoid into surrounding pulp spaces.

FIG. 10. Dog spleen, showing arteriole entering ellipsoid, passing through, and terminating in an ampulla.



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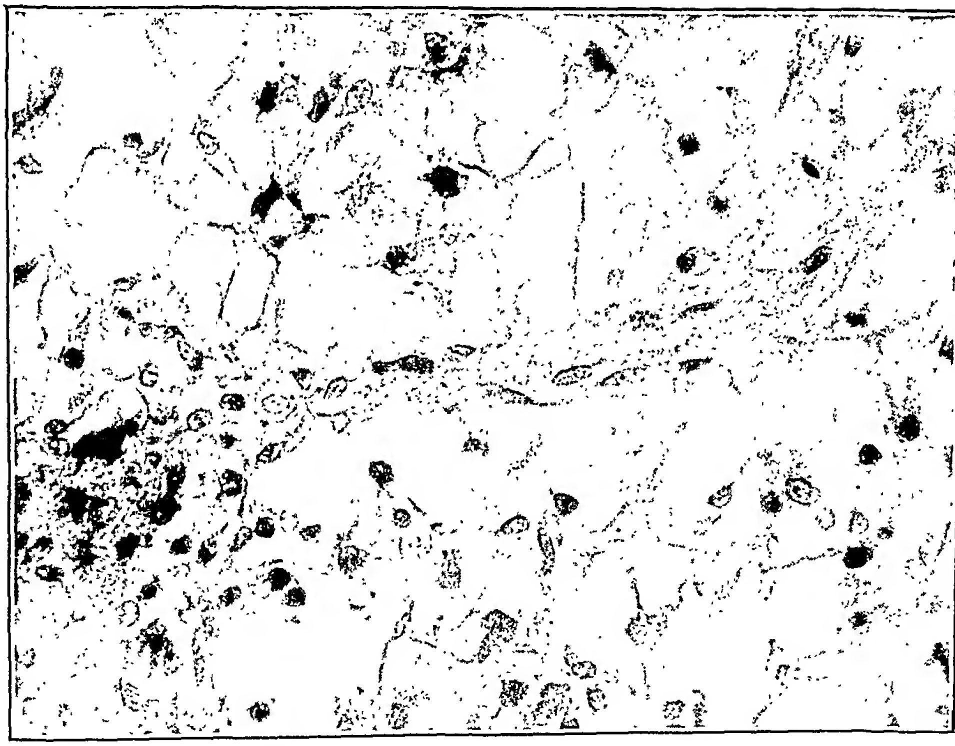

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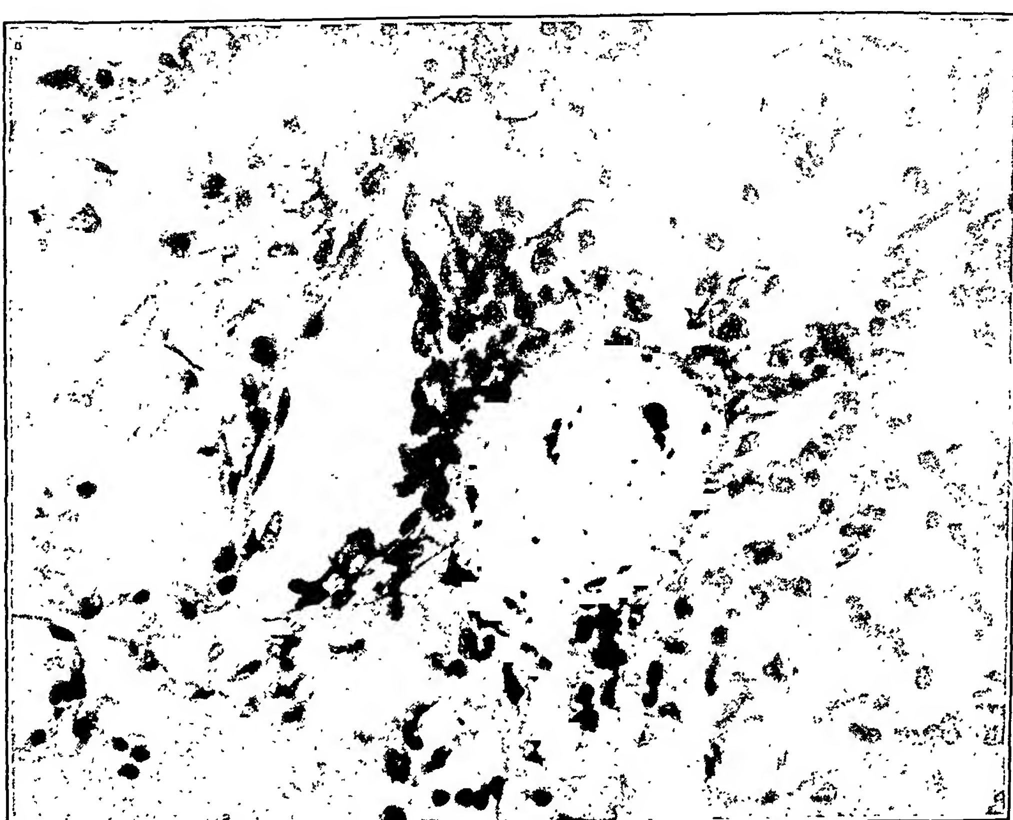

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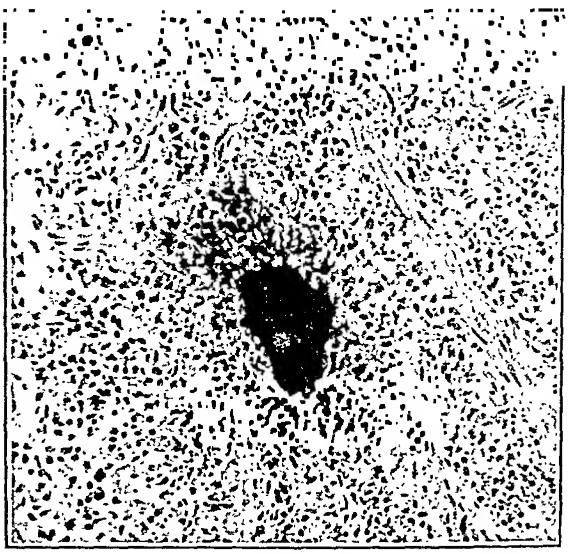

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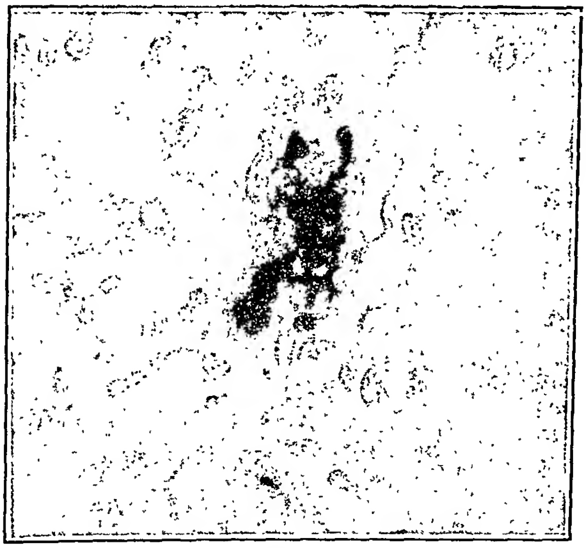

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THE ETIOLOGY OF HAVERHILL FEVER (ERYTHEMA ARTHRITICUM EPIDEMICUM) *

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Introduction

In a preliminary clinical report by Place, Sutton and Willner [1] of an epidemic that occurred in Haverhill, Mass., in January, 1926, we inserted a brief description of an organism we isolated from patients suffering with the disease. It is the purpose of this paper to give an account of the bacteriology and experimental pathology of this organism which has not previously been studied so far as we are able to determine.

Place and his collaborators have temporarily called the disease "Erythema Arthriticum Epidemicum," or Haverhill fever. They have summarized the clinical picture as being dominated "(1) by the acute onset with toxic symptoms, as chills; vomiting, malaise and headache, (2) the eruption, involving especially the extremities, of a blotchy somewhat morbilliform character with a tendency to petechiae and (3) by a multiple arthritis of varying but often severe degree." Epidemiologically, the disease was traced to raw milk distributed through stores by a farmer who handled, besides his own supply, milk from two small farms nearby.

At various stages of the disease, we made examinations of blood, throat, urine and joint fluid of patients. The blood of twenty-one patients was cultured, of which eleven were positive for the microorganism to be described (two positive twice, two positive once and negative twice, and one positive once and negative once); two were positive but contaminated and could not be purified; five were sterile; one was overheated in incubating; and four were contaminated, but apparently negative for the organism found in the positive cultures. Numerous throat cultures showed no unusual organisms. Urine cultures of three patients were too contaminated for further examination. Taps of swollen knee joints of two patients of whom one had positive blood cultures, gave pure cultures of the organism.

* Received for publication June 30, 1926.

These examinations furnished thirteen strains of which one was lost soon after recovery, leaving twelve for study, eleven from the blood and one from the knee joint. These strains are now being carried and have agreed in all respects.

Samples of milk from the twenty suspected cows on the three farms incriminated epidemiologically were cultured at the onset of the epidemic, but showed only the normal flora so far as could be determined at that time.

Numerous controls on the mediums used, technic of taking blood cultures and other sources of contamination support the reliability of the source of the organism. We believe that the microörganism recovered is the specific etiologic agent of the disease because of its high incidence in pure culture in the blood and knee joint fluid of patients sick with the disease, and because the serum of patients contains antibodies against the organism. It has not been encountered in blood cultures in any disease of our experience or to our knowledge and normal human serum does not contain antibodies against it.

We have tentatively named the organism *Haverhillia multiformis*, thereby making a new genus *Haverhillia* in the family *Mycobacteriaceae* (Chester) under the order *Actinomycetales* (Buchanan). Its classification is based on the system which is proposed by the committee on classification headed by Bergey and appointed by the Society of American Bacteriologists. The location of this organism in these divisions is due to its rod shape, usually filamentous or thread form, decided tendency to branching, irregular shapes and swellings, non-motility, requirement of complex proteins for growth and parasitism. Its classification will be discussed later.

Bacteriology

Isolation of *Haverhillia multiformis* was made by putting 5 to 10 cc. of blood from a vein, or joint fluid from a swollen knee into a flask of plain broth (veal infusion containing 0.1 per cent dextrose). At times positive cultures were exposed to two or three hours of freezing temperature in being brought to Boston, indicating resistance to cold. After incubation for twenty-four to seventy-two hours, positive cultures showed darkened blood at the bottom of the flask and on smear numerous short and long gram-negative rods. Subcultures were made in ascitic fluid broth and in clotted rabbit blood.

Identity of Strains. The thirteen strains isolated from the blood and knee joints proved to be identical by microscopic examination, culture reactions in carbohydrate broths, immunity tests with white mice and by cross agglutination and absorption tests with immune rabbit serum.

Morphology. Haverhillia multiformis appears microscopically in various forms, depending largely on the kind of medium and oxygen relations. In general, it is a rod varying in length from 2 or 3 to 10 or 15 microns and in width from 0.2 to 0.5 micron. Many rods have definitely tapering and pointed ends but the majority of the ends are rounded. Associated with other shapes in a field are often seen very short forms, appearing as coccobacilli which are single or in pairs. Often filaments and threads are seen, some extending across the microscopic field. Such filaments may be straight, curved or looped several times. Sometimes they are apparently composed of single cells, but more often they are made up of rods of varying length. Occasional filaments and threads, especially in old cultures, take the stain irregularly, showing successive fine granules in the cell body. Rarely, distinctly wavy filaments are seen. A marked characteristic is the occurrence of swellings, enlargements or knobs. These occur anywhere in the rod — terminally, subterminally or centrally. They vary in size from double to four or five times the diameter of the rod. Their shape is either perfectly round, tapering into the shape of the organism or swelling it to give a fusiform or cigar-shape. Rarely, there is seen a group of two to four such large masses strung together by a fine thread. These enlargements do not take the bacterial spore (Möller) or metachromatic granule (Albert) stains; they stain easily and intensely with the Ziehl-Neelson carbolfuchsin diluted three times.

There is a tendency to true branching which has been demonstrated by occasional, definite Y-shapes. This branching is seen only when filaments or threads occur and not among the bacillary forms.

The arrangement of the organisms in the microscopic field varies greatly. Rods and short filaments in small groups or clumps lie irregularly, either parallel, crossed or tangled together. In smears of cultures in clotted rabbit blood, they tend to lie parallel with loose rods extending from such a clump or lying free. When filaments and threads predominate, they usually lie in tangled masses, unless vig-

orously streaked out on the slide. In an unstained hanging-drop preparation, the appearance of individual rods, filaments, clumps or masses is not essentially different from that seen in stained preparations.

In experimental local lesions and in blood smears of mice fatally injected, the morphology is regular, the rod shape alone is found and the units lie in large and small clumps without definite arrangement. On the peritoneal surface of rabbits fatally injected intraperitoneally the forms are longer and somewhat tangled. No suggestion of dense colonies with peripheral finger-like projections is found under any cultural or experimental condition.

In general, it can be said that in favorable mediums and under favorable conditions, *Haverhillia multiformis* tends to be regular and rod-shaped, varying in length and occasionally filamentous, and forming compact masses with parallel units. Filaments and threads occur both on solid and in fluid mediums; all varieties of shape may be seen in one preparation. Under unfavorable conditions of oxygen tension, moisture or temperature, the morphology is irregular and bizarre with peculiar knob forms, enlargements, tapering rods and variation in size.

Staining Reactions. Haverhillia multiformis is not easily stained with the ordinary aniline dyes; young cultures are more readily stained than old ones. It is gram-negative and non-acid fast. The enlargements of the bacterium do not take the spore stain. No capsules have been demonstrated and no metachromatic granules are stained by Albert's method.

Colony. The colony in ascitic fluid or animal serum broth is white, soft and not easily broken up. It is 1 to 2 mm. in diameter, round and has the appearance of a miniature cotton-ball. When dislodged from the side of the tube, the mass slowly settles. At the bottom of a tube of fluid medium the growth appears as a grayish white layer that comes up in fine round particles upon agitation of the tube. Because of the tendency to form these clumps, the supernatant fluid is perfectly clear. No pellicle is formed.

Growth in semisolid agar containing ascitic fluid occurs as a diffuse grayish line following the course of inoculation. The colony on the surface of a solid medium is commonly discrete, but if very moist, the growth is somewhat confluent. It is 1 to 2 mm. in diameter, colorless or slightly grayish (as on chocolate agar), and trans-

parent; it is round and regular with a smooth convex surface and very fine radiating lines seen by low-power microscopic examination; it is soft, moist and homogeneous.

Motility. No motility is observed in hanging-drop or dark-field preparations.

Odor. No odor can be detected.

Cultivation. Food Requirements. It was found early in the investigation that *Haverhillia multiformis* grows under artificial conditions with considerable difficulty. No growth occurs on plain agar or in beef infusion broth to which 0.1 per cent dextrose is added. Whole sheep blood agar gives extremely scanty surface growth while on chocolate agar, the colonies are of fair size but not numerous. This organism flourishes, however, in broth to which 5 or 10 per cent defibrinated blood (sheep, rabbit or human) has been added. Fresh whole rabbit blood allowed to clot and inactivated fifteen minutes at 55 C, gives a good medium in which the bacterium grows profusely in twenty-four hours. Broth to which serum (rabbit, sheep, cow, horse or human) is added furnishes heavy growths in twenty-four hours, which when collected give material for study. Ascitic fluid broth is probably the best liquid medium for rapid growth.

The most favorable solid medium that we have found is composed of equal parts of glycerine extract of potato and infusion broth to which egg yolk is added. This is then coagulated in a slanting position in either tubes or bottles. Small amounts of plain broth added to this medium increase its value in detecting the organism in suspected sources, *e. g.*, body fluids of inoculated animals. Loeffler's coagulated serum is not a good medium for this organism. Either potato starch medium, a slice of potato over broth or coagulated egg yolk alone does not support a satisfactory growth. There is a slight growth in broth to which sterile pieces of animal tissue are added. Carbohydrates alone dissolved in broth give scanty if any growth.

The ability of *Haverhillia multiformis* to grow or live in milk was carefully studied because of the epidemiology of the disease. Large and small amounts of inoculum were put into milk treated in various ways and incubated at room temperature and 37 C for various periods of time. A study of Table 1 shows that the results were irregular but in general, milk is distinctly an unfavorable medium either for growth or viability. At room temperature, the organism does not grow and is not recoverable by subculture in a favorable medium.

In repeated tests at 37 C, it was twice seen in smears made by scraping the bottom of the tube with the loop; but ordinarily this was not the case even when such nutriment as whole blood or ascitic fluid was added to the milk. On the other hand, subcultures of a loopful of material from the bottom of tubes of straight milk were positive in some tests up to four days but not at the end of six days incubation. Cultures in milk with sheep blood or ascitic fluid added were positive in subculture but not on smear up to six days. At no time was there any indication of coagulation or acidity, the latter being tested by litmus indicator added either before inoculation or at the end of the test. Controls were always run on the viability of the cultures used in these tests.

Apparently, *Haverhillia multiformis* does not flourish in milk at room temperature or 37 C. It seems not to grow sufficiently to be seen on smear of the sediment of test tubes, but it either grows slightly or persists sufficiently to be subcultured up to four days but not thereafter.

In general, whole blood, defibrinated blood, serum, ascitic fluid, and egg yolk with potato starch seem to furnish the most favorable food constituents. After artificial cultivation for five months, this organism does not appear to be any more easily grown on ordinary laboratory mediums than at first.

Oxygen and Carbon Dioxide Relations. *Haverhillia multiformis* grows on all varieties of blood or fluid mediums in large or small amounts at atmospheric pressure of oxygen. On a solid medium, growth is slow and scanty at atmospheric pressure and slightly better in an anaerobic jar; but the most favorable condition is obtained in a sealed jar in which a candle has been burned. Replacement of all the air with carbon dioxide does not give a better growth than in the candle jar. Slants sealed with paraffin or sealing wax allow a fair but slow growth.

Temperature Relations. These relations were determined by planting fresh cultures in tubes of horse and cow serum broth, on the coagulated egg medium and in sealed tubes of clotted rabbit blood. The tubes were exposed to temperatures ranging from 10 to 55 C. The range of temperature for growth was found to be between room temperature (20 to 22 C) and 40 C. The optimum temperature is between 35 and 38 C. Heating fresh, healthy cultures in clotted rabbit blood kills the organism in fifteen minutes at 55 C. Cultures

Table 1

Milk experiments with *Haverhillia multiformis*

Temperature of incubation	Milk	Number of tests	Per cent milk	Medium added	Period of incubation	Results	
						Direct smear	Subculture
20 C.	fat-free, autoclaved	2	100	. . . .	to 13 days	repeatedly negative	repeatedly negative
	whole, fresh, unsterilized	2	100	. . . .	to 5 days	repeatedly negative	repeatedly negative
37 C.	fat-free, autoclaved	2	100	. . . .	to 13 days	repeatedly negative	repeatedly negative
	whole, fresh, unsterilized	2	100	. . . .	to 5 days	48 hrs., positive 5 days, negative	not done negative
	whole, autoclaved	2	10	plain broth	to 4 days	positive	not done
	whole, autoclaved	2	33	plain broth	to 4 days	repeatedly negative	not done
	whole, autoclaved	1	90	sheep blood	to 6 days	repeatedly negative	positive
	whole, autoclaved	1	90	ascitic fluid	to 6 days	repeatedly negative	positive
	whole, autoclaved	3	100	. . . .	to 6 days	repeatedly negative	4 days, positive 6 days, negative

of the bacterium in fluid and on solid mediums die in two to four days at 37 C. A twenty-four hour culture in serum or ascitic fluid broth lives a week to ten days in the icebox; in blood broth, about two weeks; in clotted rabbit blood, six weeks; and on the coagulated egg medium, about five to seven days. For carrying the stock cultures, clotted rabbit blood has been constantly used and the transfers have been made weekly.

Reaction of the Medium in Relation to Growth. Growth takes place in broth at pH 7.8 to which blood is added. Straight blood, presumably slightly alkaline, is a favorable medium. Broth to which ascitic fluid is added is pH 8.0 to 8.4 before inoculation and gives an excellent growth.

Moisture Relations. Moisture seems essential to the growth of *Haverhillia multiformis.* This necessitates using either a fresh solid medium, a solid medium to which a small amount of plain broth is added (*e. g.*, the coagulated egg medium), a sealed jar in which a candle is burned or an incubator with atmosphere saturated with moisture.

Reactions in Mediums. This organism causes in blood broths a darkening of the blood with some beta hemolysis that increases with incubation. Whole blood agar plates are slightly changed along the streak of growth. Clotted rabbit blood is also darkened after twenty-four hours incubation. Milk is apparently not changed but growth is so slight that a definite statement regarding the reaction cannot be made.

In order to find what carbohydrates are attacked by this organism, it was necessary to add to the carbohydrate broths a constituent that would encourage growth. Ascitic fluid was used which had a trace of a fermentable sugar in it. Therefore, controls were used to check the reactions. Sugar-free broth was employed. With the Andrade indicator certain reactions were obtained that were confirmed by other tests using suitable indicators. A limited number of carbohydrates has been tested; the findings constitute a preliminary report. The following protocol is an example of the reactions obtained after three days incubation of all tubes. A similar series of tubes showed a partial reaction at twenty-four hours and a complete reaction at seventy-two hours.

TABLE 2

The reaction of *Haverhillia multiformis* on certain carbohydrates

Carbohydrate	Carbohydrate broth with ascitic fluid		Carbohydrate broth without ascitic fluid	
	Inoculated *pH*	Uninoculated *pH*	Inoculated *pH*	Uninoculated *pH*
Dextrose	5.6	7.6	7.0	7.2
Dextrine	5.6	8.0	6.8	7.6
Glycerine	7.8	8.0	7.8	7.8
Lactose	6.6	7.4	7.0	7.4
Levulose	6.4	8.0	7.0	7.4
Maltose	6.6	7.8	7.0	7.2
Mannite	7.8	8.0	7.8	7.8
Saccharose	7.8	8.0	7.8	7.6
Starch	5.0	8.0	5.6	7.8
Control	7.8	8.0	7.8	7.8

All reactions were without the formation of gas. Duplicate tubes incubated seven days gave practically the same results. The table indicates that starch, dextrine and dextrose are vigorously attacked. The reactions with lactose and maltose are relatively weak, and there is a question whether the mild acidity obtained is due to a weak reaction or contamination with small amounts of dextrose split off in sterilizing the medium.

VIRULENCE AND IMMUNITY

The virulence of *Haverhillia multiformis* was studied by tests on white mice, guinea-pigs, rabbits, and a cow and calf.

White mice injected intraperitoneally with twenty-four or forty-eight hour cultures died in sixteen to forty-eight hours. Subcutaneous injections had fatal results but survival was longer. The amounts used varied from 0.5 cc. of a culture in straight sheep blood to the growth from a small slant (tube measuring 12 × 1.3 cm.) of the coagulated egg medium. Nothing distinctive was found in post-mortem examinations. The organism was regularly seen in direct smears and recovered in pure culture from the peritoneal fluid and heart blood. In the former site, the size is always smaller than usual, slender and with pointed ends, while in the heart blood it is thicker, with rounded ends, straight and in small clumps of six to a dozen or more. Feeding a few drops of a concentrated suspension of *Haver-*

hillia multiformis had no effect on two mice. The subcutaneous injection of a pregnant mouse killed in four days without inducing abortion.

Rabbits showed a constant resistance to this organism when injected intravenously, even large doses (*e. g.*, growth from 10 cc. serum broth) causing no symptoms and no localization of infection. Two young rabbits were injected intraperitoneally with massive doses. One died in three and the other in four days with evidences of an acute peritoneal inflammation which in one rabbit extended into the mediastinum. Direct smears of the fibrinous peritoneal exudate and cultures of this exudate in both rabbits and of the heart blood of one showed the injected organism in pure culture. A full-grown rabbit survived a proportionately larger dose injected intraperitoneally. Subcutaneous injections caused no remarkable reaction with small doses, but with a large dose one rabbit had local edema and swelling that subsided in three days leaving only a thickening of the subcutaneous tissues. Intracutaneous inoculations led to an erythematous area 0.3 to 0.5 cm. in diameter with a small yellow necrotic center; about such a lesion the skin was indurated. This reaction appeared in twenty-four to forty-eight hours and faded in two or three days.

One rabbit injected intratracheally survived without symptoms and had a sterile blood culture. Intra-articular injections into both knee joints of two rabbits caused in three to five days acute symptoms of swelling, redness, heat and loss of function; these symptoms subsided in about two weeks. Fluid drawn from such a joint in the acute stage was cloudy and on direct smear showed polymorphonuclear leucocytes, but no bacteria were found either by direct smear or culture. After complete healing, necropsy revealed no abnormality of the joints. Intratesticular injections of several rabbits produced an extreme local reaction of edema of the scrotum and swelling, redness and induration of the testicle with local elevation of temperature. Cultures but not smears of testes removed in the acute stage were usually positive for the organism injected. This reaction appeared in twenty-four hours and in four or five days the acute condition was reduced, leaving a firm nodular testicle.

Two of three rabbits injected intracerebrally died, one in twenty hours and the other in two and a half days. Death followed acute symptoms of nystagmus, lateral turning of the head and rapid,

labored respiration. Necropsy of each rabbit showed a small area of hemorrhage and necrosis at the site of puncture. The organism was seen in one but not in the other in the direct smear from the brain; it was recovered from both brains at the site of injection, and from a distant part. The heart blood yielded a pure culture of the bacterium in one instance but not in the other.

Growth from a twenty-four hour culture of *Haverhillia multiformis* in ascitic fluid broth injected subcutaneously into a pregnant rabbit did not induce abortion. Five cc. of a concentrated suspension of viable organisms administered to a rabbit by mouth had no apparent effect.

Guinea-pigs showed no reaction to intratesticular injections and only a slight local erythema was seen after intracutaneous and subcutaneous injections. Of eight guinea-pigs injected intraperitoneally, two died in three days but their death was complicated by pneumonia which probably was the important element, since heart blood cultures were negative for *Haverhillia multiformis*. One animal showed the injected organism on smear but not on culture of the peritoneal exudate; at no time were any macroscopic or microscopic granules or club forms found in the exudate. Three other guinea-pigs were found dead after ten days, two months and three months, but postmortem and culture examinations were negative and they may be considered to have died of some intercurrent disease. Feeding 2 cc. of a concentrated suspension of organisms by means of a catheter had no effect.

A *cow* and her suckling *calf* were procured to attempt to reproduce the epidemiology of the disease. We had found that patients showed a high titer of antibodies and we had demonstrated antibodies in the serum of one cow on the suspected farms. If the cow is susceptible and if we could give the experimental animal an intravenous inoculation of such a strength that it would produce an infection either generally or in the udder, she might excrete the organism in her milk. This would then be taken by the calf which also might react with the production of antibodies. If these conditions of the epidemic could be repeated, it would furnish strong evidence in support of the epidemiology.

In preliminary tests we found that the cow and calf had no antibodies against *Haverhillia multiformis* and that the organism could not be demonstrated in the milk by culture or smear of sediment.

TABLE 3

Summary of animal experiments

Animal	Number injected	Number of strains used	Route	Number recovered	Number died	Average period of survival	Findings
White mice..	22	5	Intraperitoneal	4	18	29 hours *	Injected organism in heart blood culture of 16 mice
	3		Subcutaneous	0	3	5 days	Injected organism in heart blood culture of 3 mice
	2	1	By mouth	2	0	....	No effect
Guinea-pigs .	8	5	Intraperitoneal	3	2	3 days	Peritonitis complicated with pneumonia; heart blood cultures negative
					3	10 days 2 months 3 months	Postmortem examinations and cultures negative
	2	2	Intratesticular	2	0	....	Negative
	1	1	Intracutaneous	1	0	....	Very slight erythema
	1	1	Subcutaneous	1	0	....	Slight edema and thickening
	1	1	By mouth	1	0	....	No effect
Rabbits.....	8	4	Intravenous	8	0	....	No reaction
	3	2	Intraperitoneal	1	2	3 days 4 days	Acute peritonitis and positive heart blood cultures Acute peritonitis; negative heart blood cultures
	1	1	Subcutaneous	1	0	....	Temporary edema and induration of subcutaneous tissues
	4	1	Intracutaneous	4	0	....	Small area of erythema with necrotic center
	6	1	Intratesticular	6	0	....	All signs of acute local inflammation; local cultures positive
	1	1	Intratracheal	1	0	....	No reaction
	2	2	Intra-articular	2	0	....	Acute local inflammation
	3	2	Intracerebral	1	2	$2\frac{1}{2}$ days	Local reaction; cultures positive
	1	1	By mouth	1	0	....	No effect
Cow........	1	1	Intravenous	1	0	....	Febrile reaction; no antibodies
			Subcutaneous (3 injections)			....	Temporary local swelling and tenderness followed by induration; complete and rapid healing
Calf........	1	1	Suckling	1	0	....	Taking milk from mother did not produce antibodies
			Subcutaneous (3 injections)			....	No effect

* This figure excludes one mouse that died after 5 days and whose heart blood culture showed a member of the *Bact. coli* group but not the organism injected.

We injected the cow in the jugular vein with the growth from a forty-eight hour culture in eleven 100 cc. flasks of serum broth. She had a temporary reaction, but was eating and appeared normal before the end of the day. Her rectal temperature began to rise the second day after injection, reaching 103.5 F on the third day; the temperature continued to vary from 100 to 104 F, gradually subsiding in three weeks. Blood cultures were persistently negative except for one positive culture taken the day after inoculation. Repeated milk examinations were negative. Agglutination tests of the serum from cow and calf were negative ten and twenty days after the inoculation of the cow.

In order to test further the susceptibility or resistance of the cow and calf to *Haverhillia multiformis* we injected them subcutaneously each three times with large concentrated suspensions of this organism. The calf did not react at all and in two or three days the sites of inoculation were entirely normal. The cow showed tender, swollen, subcutaneous masses at the sites of injection, but these became small and indurated in five days and in twelve days had disappeared entirely.

We were not able to set up in the cow, by the single large intravenous injection, an infection capable of producing antibodies or to infect the calf through the milk. Repeated subcutaneous injections of large doses were innocuous to both animals.

Increase and Decrease in Virulence. No increase in virulence was noted after passage through an animal. After artificial cultivation for five months, as small a dose as at the beginning of the investigation was fatal for white mice. Likewise, intracerebral and intratesticular injections into rabbits produced the same lesions in the same time as in the earlier experiments.

Immunity. Four mice recovered from sublethal and dead cultures. One such injection rendered them immune to doses of homologous and heterologous strains of *Haverhillia multiformis* fatal to control mice. This immunity was found to persist at least two months. Table 4 is a summary of these immunity experiments with white mice. Feeding did not confer protection against subsequent injections lethal for control mice.

TABLE 4

Immunity experiments with white mice

Immune Mice	Same as immunizing strain	Different from immunizing strain				Control Mice	
Mouse number	I2	I1	I2	I3	I4	Number injected	9
Times injected	3	1	2	6	6	Number died	9
Times recovered	3	1	2	6	6	Positive heart blood cultures	8

SEROLOGY

EXPERIMENTAL TESTS

Rabbits injected intravenously four to six times with increasing doses of viable organisms produced immune serums that gave positive precipitin reactions. Antigens for this purpose were prepared in two ways: (1) by growing a strain on the coagulated egg medium, incubating the growth washed off in 0.5 per cent phenol for twenty-four hours and clearing by centrifuging; and (2) by collecting the supernatant fluid of old ascitic fluid broth cultures of all strains. Both antigens diluted 1:20 gave positive precipitin reactions with immune rabbit serum and not with normal rabbit serum.

The serum of rabbits similarly immunized repeatedly agglutinated *Haverhillia multiformis* in dilutions reaching 1:5000 while normal rabbit serum did not agglutinate the same suspension above 1:20. Various antigens were used, the best being the growth from a twenty-four hour culture in ascitic fluid or serum broth resuspended in normal salt solution. Other antigens tried were fresh and old cultures from the coagulated egg medium, which were used either untreated or after repeated freezing and thawing; such antigens, however, were not so satisfactory as when prepared from the fresh cultures described. It was found that shaking the mixture of immune serum and suspension for ten minutes before incubating hastened but did not alter the reaction. This was adopted as a routine measure in all agglutination tests. Incubation at 37 C in a water bath for one hour is sufficient, but at the end of two hours the reaction is more complete. There is some danger of spontaneous clumping after two or three hours incubation so that a close observation of the salt solution and normal serum controls is necessary.

The serum of a rabbit immunized against only one of the strains reacted similarly with suspensions of all other strains (Table 5).

Absorption of antibodies from this serum both by the homologous strain and by a heterologous strain removed all agglutinins against the other strains as well (Table 6). These tests indicate further the identity of the strains recovered from the patients.

TABLE 5

Cross agglutination tests with serum of rabbit immunized against one strain

Strain	Serum of R 1–154				Control in salt solution	Serum of normal rabbit	
	1 : 100	1 : 200	1 : 500	1 : 1000		1 : 50	1 : 100
No. 150.........	3	3	2	1	0	0	0
No. 152.........	4	3	3	2	0	0	0
No. 154.........	4	4	3	2	0	0	0
No. 162.........	4	4	3	2	0	0	0
No. 187.........	4	3	3	2	0	0	0
No. 212.........	4	4	3	1	0	0	0
No. 213.........	4	3	2	1	0	0	0
No. 214.........	4	4	2	1	0	0	0
No. 256.........	4	4	3	2	0	0	0
No. 258.........	4	4	3	2	0	0	0
No. 259.........	4	4	4	2	0	0	0

Legend: Figures refer to degree of agglutination reaction; 4 = complete reaction.

TABLE 6

Absorption tests with serum of rabbit immunized against one strain

Serum of R 1–154 absorbed with strain No. 154

Strain	Absorbed serum 1 : 100	Unabsorbed serum 1: 100	Strain	Absorbed serum 1 : 100	Unabsorbed serum 1 : 100
No. 150....	0	4	No. 212....	0	4
No. 151....	0	4	No. 213....	0	4
No. 152....	0	4	No. 214....	0	4
No. 154....	0	4	No. 256....	0	4
No. 187....	0	not run	No. 258....	0	not run

Serum of R 1–154 absorbed with strain No. 256

Strain	Absorbed serum 1 : 100	Strain	Absorbed serum 1 : 100
No. 150....	0	No. 212....	0
No. 151....	0	No. 213....	0
No. 152....	0	No. 214....	0
No. 154....	0	No. 256....	0

Legend: See Table 5.

Complement fixation tests were positive with an immune rabbit serum and not with a normal rabbit serum. The antigens used were the supernatant fluids of carbolized growths from the coagulated

egg medium and of the growth in old ascitic fluid broth cultures, repeatedly frozen and thawed.

As stated before, we were unable to demonstrate agglutinins in the serum of the experimental cow injected intravenously with a large single dose of the bacterium. The calf which fed only from its mother likewise showed no agglutinins in repeated tests.

TABLE 7

Agglutination tests of patients' serum with polyvalent antigen

Patient's serum	Serum dilution 1:20	1:50	1:100	1:200	1:500
No. 150	4	4	4	3	0
No. 151	4	4	4	4	1
No. 152	3	2	1	0	0
No. 153	4	3	2	1	0
No. 164	4	3	2	1	0
No. 212	4	3	2	1	0
No. 213	4	4	3	1	0
No. 218	4	4	4	1	0
No. 256	4	3	2	1	0
No. 258	4	3	2	1	0

Immune rabbit serum control	Serum dilution 1:100	1:500	1:1000	1:5000
R 3–214	4	4	3	2

Legend: See Table 5.

CLINICAL TESTS

The serum of *patients* sick with the disease was collected at the time of the epidemic but difficulty in growing the organism recovered from them and in preparing a suitable antigen necessitated delaying the serologic tests for three months. At that time, agglutination tests were carried out with positive results on the serum of ten patients of which nine had given positive and one negative blood cultures. Two samples were received from some patients in different stages of the disease. The serum of two patients with positive blood cultures was not available. The ten serums on hand reacted similarly in three sets of experiments with polyvalent antigens prepared as for the rabbit tests above. The titers of the various serums ranged between 1:20 and 1:500 with the majority of the titers at 1:50 and 1:100. These results are given in Table 7. Control tests were made at the same time on the serum of forty normal persons. These were consistently negative in dilutions above 1:20 and only two gave weak reactions in that dilution.

The blood of the *cows* whose milk was being sold at the time of the epidemic was obtained three months later. These included the cows of the three farms and numbered fifteen. Cultivation of all specimens was negative bacteriologically for *Haverhillia multiformis*. The serums from these specimens were tested for agglutinin content in an effort to trace the source of the organism with which the patients' serum reacted. Three series of tests were run with various antigens prepared as for the tests with rabbit serum. As controls, the serum of nine cows taken at random at the local abattoir was used. The serum of one cow (cow 11) on the farm of the milk dealer agglutinated *Haverhillia multiformis* completely in the dilution of 1: 100 and partially in 1: 200. Of the other cows on the three farms and the nine control cows, the serum of a few gave a partial reaction in a dilution of 1: 20 but not above.

Nothing remarkable was noted regarding the cow that reacted positively except for a superficial lesion on one teat and another teat that dripped milk constantly. The local and state veterinarians inspected the herds on the three farms and pronounced them apparently well. Unfortunately, this cow could not be studied thoroughly because of the distance of the farm from Boston and because of the hostility of the farmer; when steps were taken to purchase the cow for study, it was learned that she had been sold to the butcher.

Microscopic Pathology

R 1–151, a young rabbit, was injected intraperitoneally with a large dose of *Haverhillia multiformis* and died three days later with gross evidence of acute fibrinous peritonitis with extension into the mediastinum and pleural cavities. A brief account of the microscopic pathology follows. *Heart:* Negative. *Lung:* Necrotic cellular débris and clumps of the organism are on the pleural surface. *Spleen:* Polymorphonuclear and endothelial leucocytes with necrotic cellular débris are found on the surface with masses of the bacterium injected seen as intertwining threads; otherwise the spleen is negative. *Liver:* Large numbers of the organism with fibrin and polymorphonuclear leucocytes are on the surfaces; there are foci of necrosis of the liver cells near the capsule; elsewhere the liver is negative. *Gastro-intestinal tract:* Many polymorphonuclear leucocytes, fibrin and masses of bacteria are on the peritoneal surface; otherwise the tract is negative. *Adrenals* and *kidneys:* Nega-

tive. *Bone marrow:* Extensive necrosis of hematopoietic cells including the megalokaryocytes is found; many of these latter cells are apparently degenerated and contain pink-staining hyaline masses in their cytoplasm.

A testicle from each of two rabbits inoculated intratesticularly was removed after forty-eight hours (R 2–256) and seven days (R7–256). Microscopically the forty-eight hour specimen shows a more acute condition, namely, an extensive necrosis of the germinal epithelium and connective tissue with fibrin in places in the necrotic material. The periphery of this necrotic area is infiltrated with polymorphonuclear leucocytes; about the lesion are many such leucocytes together with endothelial leucocytes of which large numbers contain cellular débris. The typical bacteria occur in clumps as short to medium length rods.

The other testicle (R 7–256) removed seven days after inoculation shows an area of necrotic germinal epithelium and connective tissue with fibrin and edema surrounded by an area of necrotic tissue infiltrated with polymorphonuclear leucocytes many of which are dead. This whole area is becoming encapsulated by granulation tissue showing some fibroblasts growing into the necrotic tissue; in this granulation tissue, endothelial leucocytes and lymphocytes are seen. No bacteria are found anywhere in the lesion. *Heart, spleen, liver* and *kidneys* of this rabbit are negative.

Two rabbits died following intracerebral inoculations. R 5–256 died after twenty hours and shows microscopically at the site of the inoculation a small area of necrosis in the cortex which is infiltrated with polymorphonuclear leucocytes. About the lesion are focal hemorrhages. In the necrotic tissue and extending into the surrounding area are found masses of short and medium length bacteria without irregularity of shape. Elsewhere the brain is normal.

R 4–256 died seventy hours following inoculation and microscopic examination of the organs follows. *Brain:* There is an area of necrosis in the cortex with hemorrhage and infiltration with polymorphonuclear leucocytes. Scattered around this area are small focal hemorrhages and in the necrotic tissue are seen clumps of short and medium length bacillary rods. The meninges in the vicinity of the cortical lesion show polymorphonuclear leucocytes, fibrin and dilated blood vessels. Elsewhere the brain is normal. *Liver:* Sections of this organ show focal necrosis and hydrops of cells in the center of the lobules. *Spleen* and *kidneys* are negative.

Discussion

The identification of this organism isolated from the blood and knee joint fluid of patients is a matter of some difficulty. Such a pathogenic bacterium has not been met by us and we have found no reference to it in books or literature on systematic bacteriology. The disease with which this organism was associated is unusual and has been given a distinctive name. These facts lead us to believe that it is a hitherto undescribed parasitic microörganism.

In classifying this organism, we followed the system as presented by the committee on classification appointed by the Society of American Bacteriologists.[2] It belongs, we believe, among the actinomycetes as representing that large group of microörganisms whose cells are elongated and frequently filamentous with decided tendency to branching. Swellings, clubs and irregular shapes of the cells and the lack of motility and endospores are other features common to our organism and this group of higher bacteria. We eliminated the possibility of our bacterium being *Actinomyces bovis* because it is gram-negative and forms neither granules, star-shaped masses with finger-like projections nor marked branching. The actinobacillus of Lignières and Spitz[3] received considerable consideration because of the epidemiology of Haverhill fever and the general similarity of that bacillus and our organism. But we concluded that they are different because the actinobacillus is pathogenic for guinea-pigs with the formation of granules in the pus after intraperitoneal injection, and because of the presence of radiating masses in the pus, the facility of growth in ordinary laboratory mediums including milk and the surface of plain agar, turbidity in broth with formation of a pellicle, the necessity of body temperature for growth, the adherence of the colony to solid mediums, the arrangement as a coccobacillus or diplococcus and finally the characteristic of bipolar staining. *Haverhillia multiformis* has none of these characteristics.

We believe our organism falls in the family *Mycobacteriaceae* (Chester) because of its parasitism, rod shape with frequent irregularity in form, slight or occasional branching and occasional uneven staining. The genera in this family as outlined in Bergey's Manual of Determinative Bacteriology do not include our organism because of acid-fast or gram-positive staining, obligate parasitism and inability to ferment carbohydrates. It approaches genus IV the most

closely but is distinct from it since the genus *Pfeiferella* does not attack carbohydrates and our organism does. Consequently, we propose the formation of a new genus (Genus V) with the name of *Haverhillia* and the type species as *Haverhillia multiformis*. The name is based on the town in which the disease was first studied and the most out-standing characteristic of the organism. A description of the type species follows:

> Slender, gram-negative and non-acid resisting rods staining with some difficulty; often forming threads and showing tendency toward branching; marked irregularity of form with swellings and enlargements; fermentation of some carbohydrates; in general, requiring blood or ascitic fluid for growth.

The relation of *Haverhillia multiformis* to the disease as the specific etiologic agent is based on its recovery from the blood and joint fluid from twelve of twenty-two patients suffering with the disease and on the finding of specific agglutinins for the organism. The bacterium is pathogenic for white mice and rabbits and has the characteristics of parasitism in being difficult to grow under artificial conditions and in preferring body temperature. Numerous controls on the medium in which the cultures were taken, syringes used and technic of taking the cultures prevented any possibility of this being a contaminating organism. Because of these facts, we believe that we isolated the specific etiologic agent of Haverhill fever and that it is a pathogen and not a contaminating saprophyte.

We have incomplete data on the source of the organism. Epidemiologically, the disease was traced to milk. We found, on the other hand, that under artificial conditions *Haverhillia multiformis* does not flourish in milk. We found it to live in milk for four days at body temperature but the increase was slight, if any. It is conceivable that large numbers in the milk can survive and reach humans under natural conditions. Presuming that this might be possible, we looked among the incriminated herds for sources of the organism. Milk cultures taken at the time of the epidemic were negative, but such tests are not entirely reliable. Because of certain difficulties, blood cultures of the cows were, unfortunately, not made until three months after the epidemic; at that time they were negative. Our only evidence supporting the belief that the cow is the source of the organism is the fact that the agglutinin titer of one cow was 1:100.

The tests of the serum of the other cows on the three farms and several unsuspected cows were at most only slight at the 1:20 dilution at which a reaction is negligible. The evidence involving this one cow is not to be overlooked; however, evidence must yet be presented to show that one cow could scatter sufficient organisms through a medium experimentally unfavorable for the organism and diluted with other cows' milk to produce such a widespread epidemic. Furthermore, in a necessarily limited number of experiments, we found a cow not susceptible to this organism. This problem is more suited for experimentation on a larger scale under proper facilities.

We have no direct evidence as to the mode of entry of *Haverhillia multiformis* into the human body. Human experiments were not done. Feeding animals experimentally was unsuccessful. Injection of the organism was the only means found to infect experimental animals.

The experimental pathology of *Haverhillia multiformis* shows that lesions having the characteristics of acute inflammation are produced by local injections which if not fatal go on to healing without the production of chronic conditions or abscesses. Nothing like actinomycosis or actinobacillosis is found grossly or microscopically. Polymorphonuclear leucocytes are called out in the acute stages, followed in repair by endothelial leucocytes, lymphocytes and fibroblasts. The only peculiar lesion that has been found is the change in the bone marrow in which the megalokaryocytes show necrosis with pink-staining hyaline masses in the cytoplasm.

Summary and Conclusions

A report of the bacteriology, serology and experimental pathology of a bacterium isolated from patients suffering with Haverhill fever is given. Evidence is presented to show that this organism is the specific etiologic agent of the disease. The importance of taking blood cultures in similar conditions is emphasized, as such a procedure proved of great value in this investigation.

The organism seems to belong to the order *Actinomycetales* (Buchanan) and in the family *Mycobacteriaceae* (Chester). Since it has characteristics incompatible with the genera of this family, a new genus is proposed and the organism has been tentatively named *Haverhillia multiformis*.

No direct evidence of the source of the specific bacterium in the epidemic has been found, although the serum of a cow in the suspected herds contained agglutinins against the organism. Experimental data to explain the mode of entry into the human body were not obtained.

Credit is due Miss Marion E. Lamb of this laboratory for first demonstrating this bacterium in the blood cultures.

We are extremely grateful to Dr. F. B. Mallory for taking the photomicrographs. A note on the technic follows.

Wellington ortho process contrast plates were employed in making all negatives. The photomicrographs were taken with a Zeiss outfit supplied with a tungsarc light. A 2 mm. apochromatic oil immersion objective and a No. III homal ocular were used with a Wratten and Wainwright color screen B. The organisms were stained deeply with gentian violet.

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DESCRIPTION OF PLATES

Plate 71

Fig. 1. Twenty-four hour culture in ascitic fluid broth incubated and photographed in slanting position. × 2.

Fig. 2. Forty-eight hour culture in ascitic fluid broth. Contents of flask poured carefully into flat-bottomed glass dish and photographed. × 2.

Plate 72

Fig. 3. Twenty-four hour culture in clotted rabbit blood. Note regularity of shape and parallel arrangement. × 2000.

Fig. 4. Twenty-four hour culture on the coagulated egg medium; incubated in sealed jar in which candle was burned. Note frequent streptobacillary and coccobacillary forms. × 2000.

Plate 73

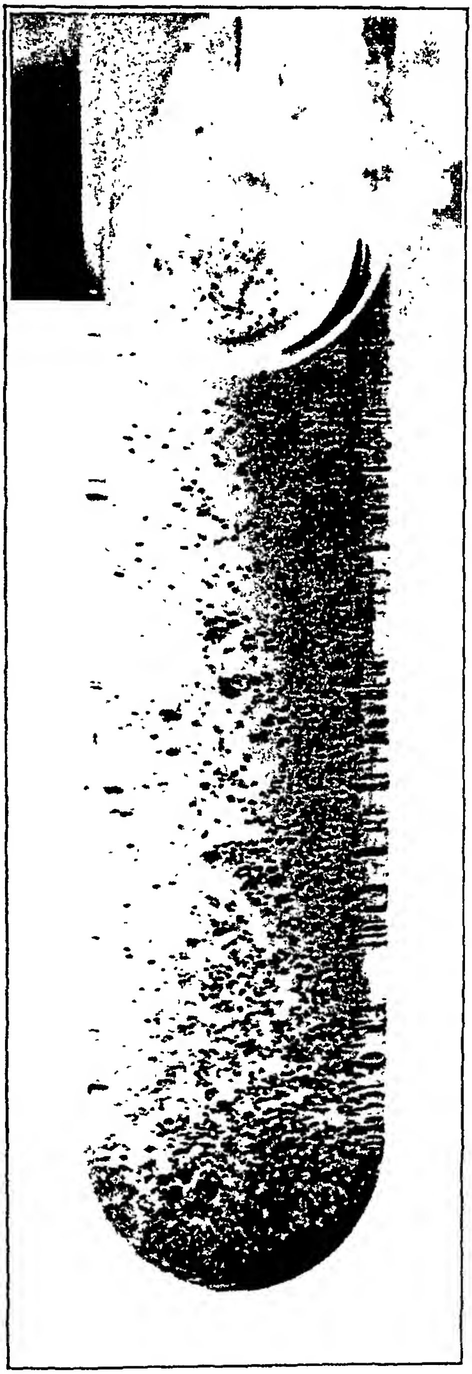
Fig. 5. Another preparation of the same culture and incubation as Fig. 4; showing more irregularity of shape and size with occasional fusiform and swollen rods. × 2000.

Fig. 6. Eight day culture on the coagulated egg medium; incubated in sealed jar in which candle was burned. A tangle of threads and filaments showing occasional thick forms and Y-shapes suggesting branching. × 2000.

PLATE 74

FIG. 7. Another field of the same preparation as Fig. 6. Irregular forms and large swellings in threads and filaments; occasional branching form. × 2000.

FIG. 8. Another preparation of the same culture and incubation as Fig. 6. Marked irregularity of shape and size with some filaments staining irregularly. × 2000.



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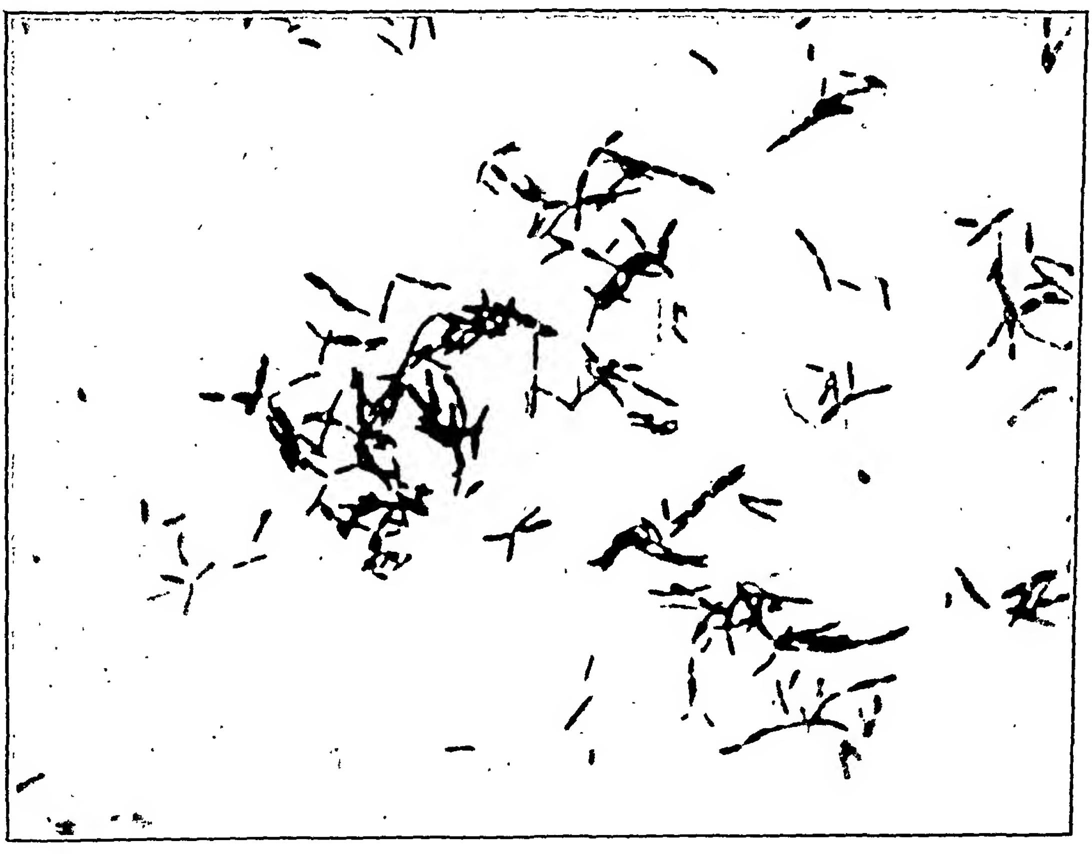

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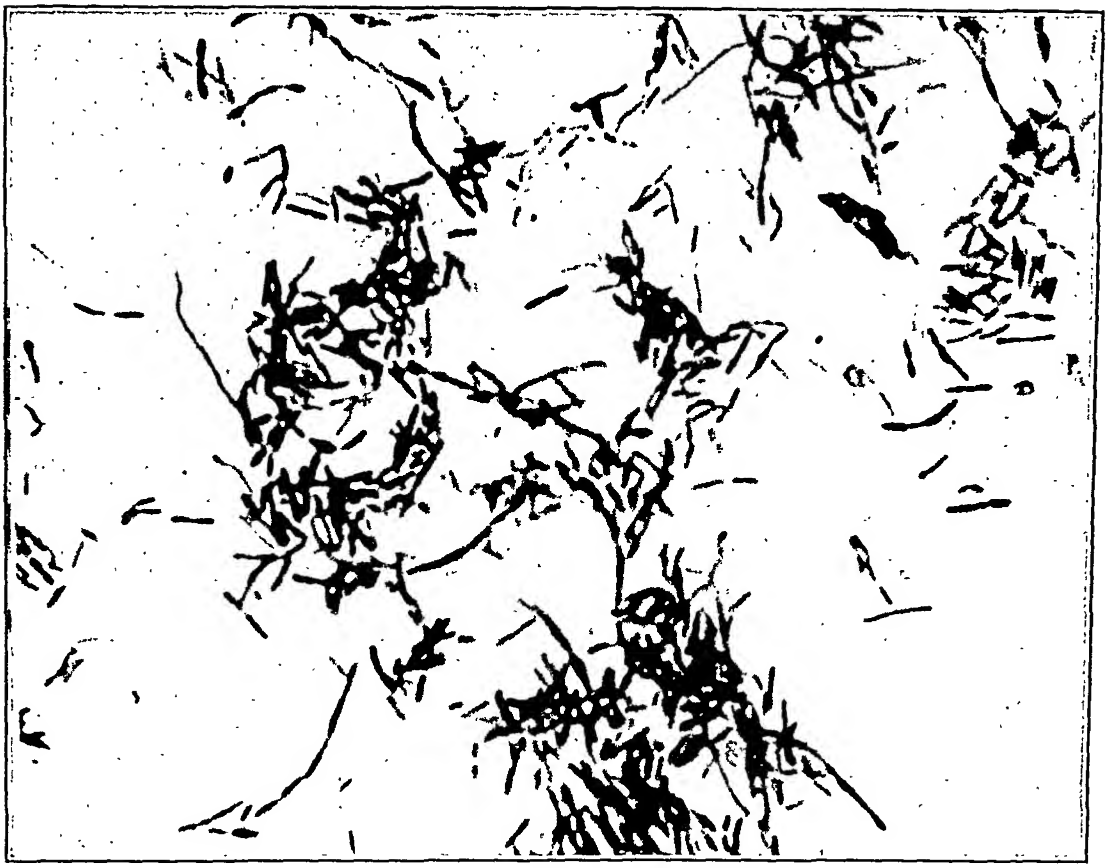

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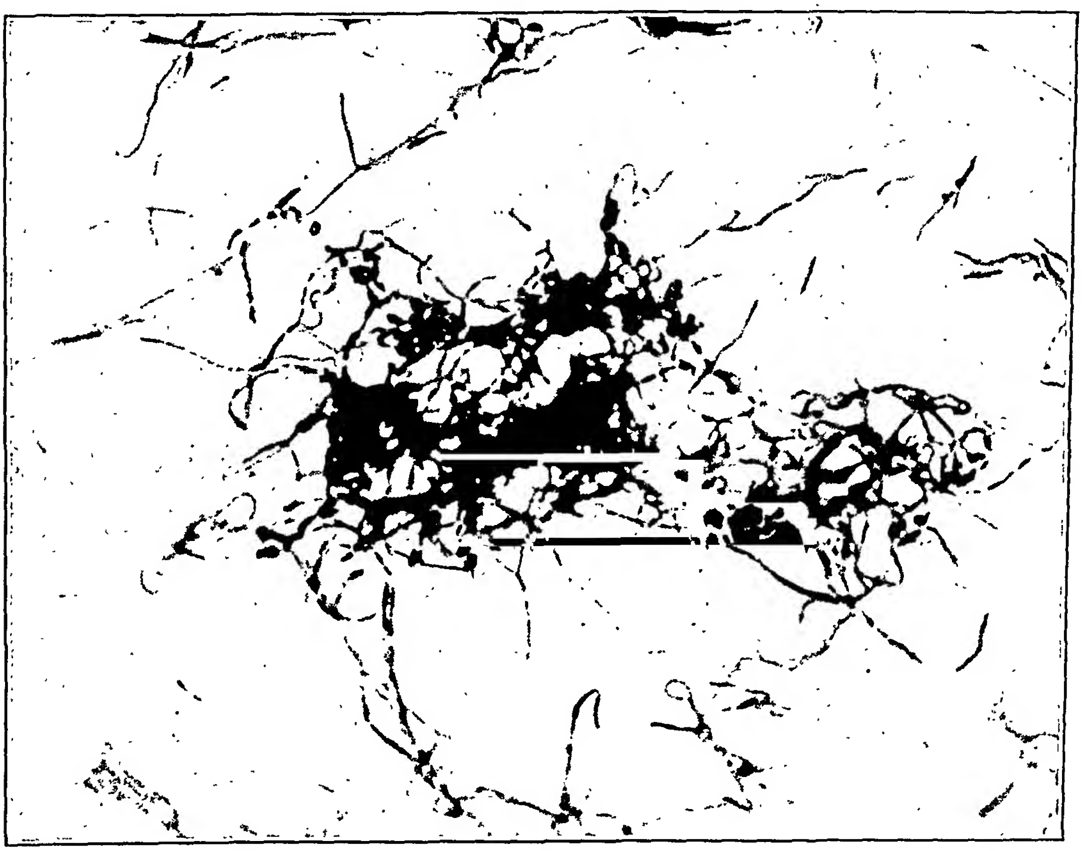

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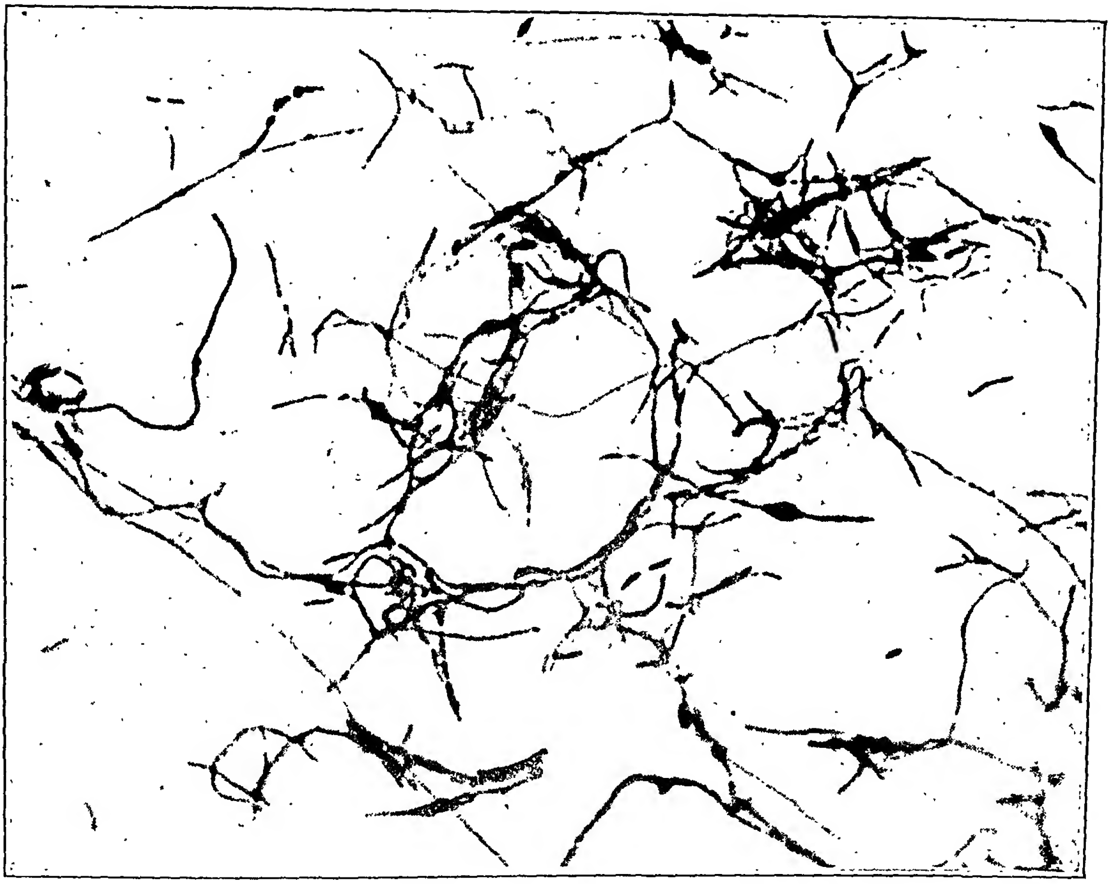

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8

SO-CALLED "ENDOTHELIAL BLOCKADE" WITH COLLARGOL

AN IMMUNOLOGIC AND HISTOLOGIC STUDY *

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Much has been written in recent years on the results produced by the injection of various colloidal suspensions, with special attention devoted to the effect on the so-called reticulo-endothelial system. Jungeblut and Berlot [1] in one of their recent publications give a good review of the literature, a summary of which would show somewhat conflicting reports as to the effect of so-called "endothelial blockade." These two authors, utilizing injections of India ink, delayed antitoxin formation in guinea-pigs; [1] in a second paper,[2] they claimed to have shown diminution in complement following similar injections. Gay and Clark,[3] employing trypan blue, demonstrated diminished antibody production in rabbits, while Lewis and Loomis [4] found the converse to be true; this discrepancy the latter authors reconciled on the basis of the general physiologic law that substances which are stimulating in certain doses are depressing in larger amounts. Isaacs,[5] working with trypan blue, apparently found diminished hemolysin production in guinea-pigs but no protection against anaphylactic shock. Howell and Tower [6] found that nine to nineteen injections of 2 to 6 cc. each of saccharated iron oxide did not influence typhoid agglutinin titers in rabbits. Some of these workers use the term "endothelial blockade" without justification in our opinion, as they failed to control their results histologically; this is especially true as regards trypan blue for this dye is easily diffusible and is found in epithelium in as large if not larger amounts than in endothelium. It likewise persists in considerable amounts for a long time in the plasma.

We decided to attempt work along these lines, using for our injections collargol, a substance that is difficultly diffusible, that is

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taken up practically exclusively by endothelium and allied cells and that is not easily eliminated. We also planned a careful histologic study in order to determine the location and amount of collargol taken up by the tissues and also to see if any correlation between the morphologic and immunologic findings was possible. The collargol used in these experiments was that manufactured by the Heyden Chemical Works and distributed by Shering and Glatz, New York. It was used either in 1 per cent or 2 per cent suspension in sterile distilled water and filtered immediately before use. We used both guinea-pigs and rabbits.

Experiments with Guinea-pigs

We first attempted to inject our guinea-pigs intracardially with collargol but had to discontinue this method since such injections proved fatal, apparently due to the fact that collargol caused the formation of intracardiac clots. We then used the ear veins and, if necessary, the leg veins and jugulars. We found the largest dose which could be safely employed to be 1 cc. of a 1 per cent suspension. As antigen, we used in our first and second series hen blood, for we hoped to be able to study phagocytosis of the nucleated erythrocytes; this however proved unsatisfactory and so in our third series we used sheep blood.

The first series consisted of five guinea-pigs, three serving as controls. The collargol guinea-pigs were injected as follows.

Guinea-pig 1 received a total of six injections of 1 cc. each, in a period of nine days. On the thirteenth day after the first injection, it received 3 cc. of 16 per cent washed and packed hen cells subcutaneously and a like amount intraperitoneally. Its serum was titrated on the ninth and twenty-second day following this injection. The animal was killed at the time of the last bleeding and its organs preserved for study.

Guinea-pig 2 was given a total of four injections of 1 cc. each in a period of five days. On the sixth day it was given 4 cc. of 16 per cent washed and packed hen cells intravenously. It was found dead the next morning showing grossly an enlarged spleen and fatty liver. The histologic picture will be described below.

Two other guinea-pigs received 2 cc. of 16 per cent washed and packed hen cells intravenously and were killed in four and twenty-seven hours respectively for histologic purposes.

A control guinea-pig received the same amount of hen cells as Guinea-pig 1 and its serum was titrated at the same time intervals.

The results of the titrations of these serums at the twenty-second day bleeding are given in Tables 1 and 2, Table 1 giving the agglutinin and Table 2 the hemolysin titers.

TABLE 1

Antigen: 2 per cent hen blood.
Incubated 2 hours at 37.5 C, then overnight in icebox.

Serum Dilutions	Guinea-pig 1	Control
1:10	++++	++++
1:20	++++	++++
1:40	+++	++++
1:80	–	++++
1:160	–	+++
1:320	–	–

TABLE 2

Antigen: 2 per cent washed and packed hen cells, 0.25 cc.
Complement: 0.3 cc. 1:10 guinea-pig serum.
Total volume: 1.25 cc.
Incubated 1 hour at 37.5 C.

Serum	Guinea-pig 1	Control
0.05	C	C
0.025	C	C
0.01	o	C
0.005	o	C
0.0025	o	o
0.0001	o	o

(C = complete hemolysis; P = partial hemolysis;
o = no hemolysis)

The second series consisted of five collargol guinea-pigs and two controls. The injections each of 1 cc. are given below:

Guinea-pig 3 — six injections in the course of ten days
" " 4 — five injections in the course of nine days
" " 5 — six injections in the course of eleven days
" " 6 — four injections in the course of four days
" " 7 — five injections in the course of five days

All animals received in the jugular vein 3 cc. of defibrinated hen blood, diluted 1:3, four to six days following their last collargol injection. Guinea-pigs 3 and 6 were killed six hours later for histologic

study. The others, including two normal animals that were similarly injected with hen blood, were bled from the heart on the ninth and twenty-second day and their serums titrated. Guinea-pig 5 was killed on the twenty-fifth day for histologic purposes. On the twenty-sixth day Guinea-pigs 4, 7 and the two controls were given in the jugular veins 3 cc. of defibrinated hen blood, diluted 1:3. The results are given below, Table 3 showing the agglutinin titer, Table 4 the precipitin titer, both on the twenty-second day, and Table 5 the shock experiments on the twenty-sixth day.

TABLE 3

Antigen: 1 per cent washed and packed hen cells.
Incubated: 2 hours at 37.5 C, overnight in icebox.

Serum Dilutions	Guinea-pig 4	Guinea-pig 7	Control 1	Control 2
1: 10	++++	++++	+++	+++
1: 20	++++	++++	–	–
1: 40	++++	++++	–	–
1: 80	++++	+++	–	–
1: 160	+++	–	–	–

TABLE 4

Antigen: varying dilutions hen serum.

Antigen Dilutions	Guinea-pig 4	Guinea-pig 7	Control 1	Control 2
1: 2	+	–	*not done*	*not done*
1: 5	±	–	*not done*	*not done*
1: 10	–	–	++	++
1: 20	*not done*	*not done*	++	++
1: 50	–	–	++	++
1: 100	–	–	+	+
1: 500	*not done*	*not done*	–	–

TABLE 5

Injected in jugular vein with 3 cc. hen blood, diluted 1: 5.

Guinea-pig	Result	Remarks
4	Survived	Showed anaphylactic symptoms immediately after injection but recovered.
7	Survived	Same as above.
Control 1	Dead in 4 minutes	Typical anaphylactic symptoms and necropsy findings.
Control 2	Dead in $3\frac{1}{4}$ minutes	Same as Control 1.

The collargol guinea-pigs show about eight to sixteen times the agglutinin content of the controls, whereas the converse is true as regards the precipitins where the collargol animals show one-twentieth to one-hundredth as great a titer. In the shock experiments, the results are very clean out; the two controls died of typical shock in three and one-fourth and four minutes respectively whereas the collargol guinea-pigs survived.

The third series was made up of five collargol guinea-pigs and two controls. Two of the collargol guinea-pigs unfortunately died before the completion of the experiment. They were injected as follows.

Guinea-pig 8 — 6 cc. collargol in seven days, then 3 cc. defibrinated sheep blood, diluted 1:3, intravenously, followed by 3 cc. more of collargol in four days.
Guinea-pig 9 — 5 cc. collargol in seven days, then 3 cc. defibrinated sheep blood intravenously, diluted 1:3, followed by 3 cc. more of collargol in six days. This guinea-pig died following the last collargol injection.
Guinea-pig 10 — 3 cc. collargol in three days; death after last injection.
Guinea-pig 11 — 6 cc. collargol in seven days, sheep blood as Guinea-pig 8, then 3 cc. collargol in four days.
Guinea-pig 12 — 4 cc. collargol in seven days, sheep blood as Guinea-pig 8, then 1.6 cc. collargol in four days.
Controls 1 and 2 — 3 cc. defibrinated sheep blood, diluted 1:3.

The collargol survivors and the controls were bled on the fourteenth and twenty-first day following the injections with sheep blood and their serums titrated for antibodies. On the twenty-second day, they were tested for sensitization. Table 6 gives the agglutinin titers on the fourteenth day, Table 7 the same on the twenty-first day, Table 8 the precipitin titers on the twenty-first day and Table 9 the shock experiments on the twenty-second day.

TABLE 6

Antigen: 1 per cent washed and packed sheep cells.
Incubated: 2 hours at 37.5 C, overnight in icebox.

Serum Dilution	Guinea-pig 12	Guinea-pig 8	Guinea-pig 11	Control 1	Control 2
1:4	+++	++	+	++++	++++
1:10	+++	+	−	++++	+++
1:20	++	−	−	+++	++
1:40	±	−	−	+++	+
1:80	−	−	−	±	±
1:160	−	−	−	−	−

TABLE 7

Antigen: 1 per cent washed and packed sheep cells.
Incubated: 2 hours at 37.5 C overnight in icebox.

Serum Dilutions	Guinea-pig 12	Guinea-pig 8	Control 1	Control 2
1:4	++++	+++	*not done*	*not done*
1:10	++++	+++	++++	++++
1:20	++++	++	++++	++++
1:40	+++	−	++++	+++
1:80	++	−	++++	+++
1:160	−	−	+++	++
1:320	−	−	+++	−

TABLE 8

Antigen: varying dilutions sheep serum.

Antigen Dilutions	Guinea-pig 12	Guinea-pig 9	Control 1	Control 2
1:2	+	−	−	+
1:10	−	−	++	−
1:50	−	−	+	−

TABLE 9

Injected in jugular veins with 3 cc. sheep blood, diluted 1: 3.

Guinea-pig	Result	Remarks
12	Died in 45 minutes	Typical anaphylactic symptoms following injection, with respiratory distress until death.
8	Survived	Very mild symptoms.
Control 1	Dead in 54 minutes	Typical anaphylactic symptoms following injection, with respiratory distress until death.
Control 2	Dead in 4½ minutes	Typical anaphylactic death.

In this series, the collargol guinea-pigs showed a lower agglutinin titer than the controls at nine days; at this time none showed demonstrable precipitins. On the twenty-first day, the collargol guinea-pigs again were lower in their agglutinin content. Guinea-pig 12, which received a total of 5.6 cc. collargol, shows less difference from the controls than Guinea-pig 8 which received a total of 9 cc. As regards precipitins, Control 1 shows more than either collargol guinea-pig, whereas the titer of Control 2 is about the same as Guinea-pig 12 but greater than Guinea-pig 8. In the shock experiments, the two controls died, Control 1 in fifty-four minutes and Control 2 in four and one-half minutes (the slow death of Control 1 may have been due to its high content of precipitins). Guinea-pig 8 survived. Guinea-pig 12 died in forty-five minutes; the death of this

animal was not surprising for, as mentioned above, it had received considerably less collargol than Guinea-pig 8 and its anti-body titer throughout had more nearly approached the controls.

Discussion

We realize that our number of animals is small; its size, however, was due to the unavoidable difficulties of repeatedly injecting guinea-pigs intravenously with a substance such as collargol for if any of it escapes into the surrounding tissues, the veins in that region are useless for reinjection. Also the time required for the injections was great and unfortunately we had but a limited amount at our disposal. However, the results taken as a whole are sufficiently suggestive to be of some value.

Summarizing then, two facts stand out: the effect of collargol on circulating antibody formation and on anaphylactic shock. We will discuss these two facts in greater detail. The precipitin titer was determined in the second and third series and in both these series it was definitely lower in the collargol-injected guinea-pigs than in the controls. The agglutinins on the other hand showed no such relation, for in the first and third series they were lower in the collargol guinea-pigs than in the controls, while in the second series they were higher. Thus it appears that while collargol may either depress or stimulate agglutinin production, it depresses precipitin formation. Such facts suggest the thought that perhaps different cells or mechanisms are involved in the formation of antibodies against soluble antigens, such as serums, and against particulate antigens, such as blood cells. However, the question is extremely complicated and requires much further study.

The second point under discussion, the effect of collargol on anaphylactic shock, is interesting but likewise is difficult to explain. Three of the four collargol guinea-pigs survived, the animal which died being the one that received the least collargol. The explanation of this apparent protection is very complicated and cannot be simply "endothelial blockade," because the effect of repeated injections of such a substance as collargol means repeated injections of a substance primarily toxic in itself and furthermore of a foreign protein, egg albumen, and a heavy metal, silver; any or all of these factors may be the primary cause and obviously no deduction is justified at the present time.

Experiments with Rabbits

Seven rabbits were used in the first series of experiments — five were injected with collargol and two were used as controls. The five were injected as follows:

They were given intravenous injections daily of collargol, the first four injections of 1 per cent, thereafter of 2 per cent. After they had received an amount corresponding to 22 cc. of 1 per cent collargol, they were given intravenously three injections on alternate days of defibrinated hen blood, diluted 1:3 (1 cc., 2 cc. and 3 cc.); the two controls were similarly injected. Two of the collargol rabbits were killed three and one-half hours after the last injection of hen blood for histologic study. The injections of collargol were continued, 12 cc. of 1 per cent suspension being given during the period of immunization, and all animals were bled on the seventh day following the injection with hen blood and their serums were titrated.

The results of the agglutinin titrations are given in Table 10 and of the precipitin in Table 11.

Table 10

Antigen: 1 per cent packed hen cells.
Incubated: 2 hours at 37.5 C, overnight in icebox.

Serum Dilutions	R 1	R 2	R 3	Control 1	Control 2
1:10	++++	++++	++++	++++	++++
1:100 ...	++++	++++	++++	++++	++++
1:200 ...	++++	+++	++++	+++	+++
1:400 ...	+++	++	+++	++	+
1:800 ...	++	–	++	–	–
1:1600 ..	–	–	–	–	–

Table 11

Antigen: varying dilutions hen serum.
Incubated: 1 hour at 37.5 C.

Antigen Dilutions	R 1	R 2	R 3	Control 1	Control 2
1:10.........	*not done*	*not done*	*not done*	*not done*	++++
1:100........	++++	+++	++	++++	++++
1:1000.......	++++	++	–	+++	++++
1:5000.......	++	+	–	++	++
1:10,000.....	+	–	–	+	+

The above tables show that one of the collargol rabbits, R 3, had a precipitin titer one-hundredth as great as the other collargol rabbits

and the controls, whereas in its agglutinin titer it was one of the two highest. In the agglutinin titration, two of the collargol rabbits are about double the other rabbits.

One more collargol injection of 4 cc. of a 1 per cent suspension was given on the day following the bleeding, bringing the total amount given to 38 cc. The animals were then allowed to rest until the fifteenth day following the injection with hen blood; at this time, they were again bled and their serums titrated. Table 12 gives the agglutinin titers at this time, Table 13 the hemolysin titers and Table 14 the precipitin titers.

TABLE 12

Antigen: 1 per cent washed and packed hen cells.
Incubated: 2 hours at 37.5 C, then overnight in icebox.

Serum Dilutions	R 1	R 2	R 3	Control 1	Control 2
1:200	++++	−	+++	−	−
1:400	+++	−	−	−	−
1:800	+	−	−	−	−
1:1600	−	−	−	−	−

TABLE 13

Antigen: 0.25 cc. 1 per cent washed and packed hen cells.
Complement: 0.5 cc. 1: 10 guinea-pig serum.
Serum: 0.5 cc. varying dilutions.
Total volume: 1.25 cc.
Incubated: 1 hour at 37.5 C.

Serum Dilutions	R 1	R 2	R 3	Control 1	Control 2
1:500	C	C	C	C	C
1:1000	C	C	C	C	C
1:2000	C	P	C	P	P
1:4000	P	o	P	o	o

(C = complete hemolysis; P = partial hemolysis; o = no hemolysis).

TABLE 14

Antigen: varying dilutions of hen serum.
Incubated: 1 hour at 37.5 C.

Antigen Dilutions	R 1	R 2	R 3	Control 1	Control 2
1:500	+++	+++	+++	+++	+++
1:1000	++	++	++	++	++
1:5000	+	+	+	+	+
1:10,000	−	−	−	−	−

As at the seventh day, R 1 and R 3 were the highest in agglutinin and hemolysin titers. The precipitin titers of all were identical.

Eight days following the fifteenth day bleeding, two of the rabbits were again started on daily injections of collargol. The third rabbit, R 3, was injected intravenously with a heavy suspension of human tubercle bacilli. It is interesting to note that the first injection of collargol in R 1 and R 2 at this time produced symptoms of rather severe shock, probably to be accounted for by the fact that they had become sensitized to the egg albumen in the collargol. When R 1 and R 2 had received an additional amount of collargol equivalent to 32 cc. of 1 per cent collargol, they received on alternate days three intravenous injections (2 cc., 3 cc. and 3 cc.) of sheep blood, diluted 1:3. At this time, two rabbits that were being injected with trypan blue were added to the experiment. They had been given daily injections of 1 per cent trypan blue and at the time of being injected with the sheep blood along with the other rabbits had received 38 cc. of a 1 per cent suspension of trypan blue in the course of ten days. The collargol injections were continued until they had received since the beginning of the experiment a total amount equivalent to 78 cc. of 1 per cent collargol. One trypan blue rabbit had died and the other had received a total of 46 cc. of 1 per cent trypan blue suspension. They and the controls and the trypan blue rabbit were bled on the tenth day and the twenty-first day following the injection of sheep blood. Table 15 gives the agglutinin titers and Table 16 the precipitin titers, all at the tenth day. Table 17 gives the agglutinin titers and Table 18 the precipitin titers on the twenty-first day.

TABLE 15

Antigen: 1 per cent washed and packed hen cells.
Incubated: 2 hours at 37.5 C, then overnight in icebox.

Serum Dilutions	R 1	R 2	T. Blue	Control 1	Control 2
1:100	++	++	++++	+++	+++
1:200	++	++	++++	++	++
1:400	++	++	+++	++	++
1:800	±	+	+++	±	++
1:1600	−	−	++	−	±
1:3200	−	−	+	−	−

TABLE 16

Antigen: varying dilutions of sheep serum.
Incubated: 1 hour at 37.5 C.

Antigen Dilutions	R 1	R 2	T. Blue	Control 1	Control 2
1:10........	+++	*not done*	*not done*	*not done*	*not done*
1:100.......	±	+++	++++	++++	++++
1:500.......	–	++	+++	+++	++++
1:1000......	–	++	++	++	++
1:5000......	–	–	+	±	+
1:10,000.....	–	–	–	–	–

TABLE 17

Antigen: 1 per cent washed and packed sheep cells.
Incubated: 2 hours at 37.5 C and then overnight in icebox.

Serum Dilutions	R 1	R 2	T. Blue	Control 1	Control 2
1:100.......	+++	+++	++++	++++	++++
1:200.......	+++	++	++++	+++	+++
1:400.......	++	+	++++	+++	++
1:800.......	++	–	+++	++	–
1:1600......	+	–	++	–	–
1:3200......	–	–	++	–	–

TABLE 18

Antigen: varying dilutions of sheep serum.
Incubated: 1 hour at 37.5 C.

Antigen Dilutions	R 1	R 2	T. Blue	Control 1	Control 2
1:10	++	++	*not done*	*not done*	*not done*
1:100	±	±	++++	+++	+++
1:1000	–	–	++	++	++
1:5000	–	–	+	+	±
1:10,000...	–	–	±	±	–

At ten days, the agglutinin titers of the collargol rabbits were practically the same as of the controls whereas that of the trypan blue rabbit was somewhat higher. As regards precipitins, both collargol rabbits were lower than the controls and the trypan blue rabbit; this was especially true of R 1 which showed one-fiftieth the titer.

On the twenty-first day, the trypan blue rabbit was again the highest with no marked difference between the collargol and control rabbits. The two collargol rabbits at this time were again by far the lowest in precipitin titer.

On the twenty-sixth day, Control 1 and R 1 were injected intravenously with 4 cc. of sheep blood, diluted 1: 2.5. They both showed

slight symptoms of shock but recovered. R 2, Trypan blue and Control 2 were then injected intravenously with 6 cc. of sheep blood, diluted 1: 2.5. All three showed severe symptoms of shock and died in three, eight and eight minutes respectively.

Discussion

The only striking difference between our collargol rabbits and the controls was the difference in precipitin titer. In the former this was consistently and markedly lower than in the latter, whereas the agglutinin and hemolysin titers were slightly higher. It will be recalled that this same difference in precipitin titer was found in the preceding experiments on guinea-pigs and this finding in two different species of animals would appear to confirm its importance. The explanation of this fact is difficult unless one can conceive, as suggested above, that soluble and particulate antigens are handled in different ways in the body. In regard to the rabbit treated with trypan blue, it showed consistently somewhat higher agglutinins than the collargol and control rabbits, but no difference in precipitin titer. This is somewhat surprising in view of the amount of trypan blue it received, which was comparable to that used by Gay and Clark.[3]

Histologic Examination of Guinea-pig Tissues

(Throughout this description of the histologic pictures in the guinea-pigs and rabbits, the word "macrophages" is used to denote the large, mononuclear phagocytes that are not fixed tissue cells; this term is used instead of the more definite one of "monocyte," "clasmatocyte" or "endothelial leucocyte" as we have no proof of the exact type of cell we are describing.)

In describing the tissues, in each instance the picture during or immediately after the collargol injection is given, then the findings several days or weeks after the cessation of the collargol injections. Each organ examined will be taken up in turn. The results represent the study of several animals in each group.

Zenker's fluid, of course, constitutes our best routine fixative for histologic work. It was used throughout this study. But Zenker's fluid so alters the collargol that the latter is no longer black or dark brown but becomes light yellow; after routine phosphotungstic acid hematoxylin it stains yellow; with eosin-methylene blue it is

green. Tissues are easily studied even with the collargol so altered but for photographic purposes the silver must be suitably blackened, One of us has devised a method for so blackening the phagocytized collargol. Sections are brought as usual through the iodine and to water; they are then treated for five minutes in a 1 per cent gold chloride solution; afterwards they are washed three to five minutes in tap water, rinsed in distilled water and treated with equal parts distilled water and stock mixture consisting of solution ammonium sulphydrate (Merck) 1 part, alcohol 3 parts. They should remain three to five minutes, following which they are washed fifteen minutes and counterstained with basic fuchsin (0.2 per cent in 50 per cent alcohol). Dehydrate, clear and mount in balsam. The method is empirical, the exact chemical changes being obscure.

Lung. In the early stages, there is a small amount of collargol present and this is contained in macrophages in the capillaries scattered throughout the lung. In the late stage, the collargol is apparently diminished in amount and what little is present is in macrophages which are in lymphatics or in the lymphoid tissue. At no time is any collargol seen in the fixed endothelium.

Spleen. In the animals killed early, the collargol is in macrophages scattered throughout the pulp and to a slight extent in the germinal centers. There is a considerable amount of collargol. In the later stages, the organ exhibited varying degrees of sclerosis (Fig. 1). The collargol is distributed in the same manner as in the early animals but apparently is diminished. In some guinea-pigs, there are varying degrees of toxic reaction and lymphoid hyperplasia but as these changes are likewise seen in the controls they cannot be considered due to the collargol.

Liver. In the early stages, the collargol occurs in the fixed endothelium lining the sinusoids throughout the organ. In the later stages, the picture is quite different. Here, the collargol is found in focal collections of macrophages, some of which are multinucleated; several of these collargol-containing macrophages are seen in mitosis; in these foci of macrophages there often are necrotic cells, and collected about these foci are collargol-free macrophages and polymorphonuclear leucocytes; some of these latter contain collargol. The collargol is likewise in macrophages in the portal connective tissue.

Kidney. No collargol is seen in either the early or late stage.

Bone Marrow. The findings in the early and late stages are essentially the same. The collargol occurs in macrophages scattered throughout the tissue. None is seen in fixed endothelium. The marrow cells in the majority of animals show myeloblastic rather than erythroblastic activity.

Lymph Node. Only a few of these are examined and in them there is a small amount of collargol in macrophages in the sinuses and in the germinal centers.

Summary. In the guinea-pig, collargol is taken up in the largest amounts in the liver, spleen and bone marrow; very little occurs in the lung and none in the kidney. The only organ in which collargol can be found in fixed endothelium is the liver and even in this organ this is more true of the early than the late stages for in the late stages much of the collargol is in macrophages; these macrophages of course may be the same cells as the fixed endothelial cells which, after being filled with collargol, have become mobile instead of remaining sessile. That collargol is to a certain extent toxic is evidenced by the fact that in the late stage necrotic macrophages with a reaction about them are found in the liver. The apparent diminution in amount of collargol in the lung and spleen in the late stages as compared to the early stages may be accounted for in four ways: (1) the collargol has been excreted; (2) while the number of collargol-containing cells is decreased, the amount in each cell is increased; (3) the cells containing collargol have migrated to some other region; and (4) the collargol has been dissolved and taken up by some other organ.

Histologic Examination of Rabbit Tissues

Lung. In the early stages, the collargol occurs in the fixed endothelium of the capillaries and in macrophages in the capillaries and lymphatics. In the later stages, it is found in macrophages which are collected in foci in the capillaries, in the lymph nodules and in chronic inflammatory foci in the alveolar walls and the alveoli. Also some is found in macrophages in the walls of the larger blood vessels beneath the lining endothelium. None is seen in fixed endothelium.

Spleen. Early, the collargol is in macrophages scattered throughout the pulp and to some extent at the periphery of the germinal centers (Fig. 2). Many of these collargol-containing macrophages also contain nuclear débris. (The spleens of two animals killed six

and one-half hours after their final injections of collargol show collargol in the form of a granular, metallic-appearing material free in the sinuses and in the fixed endothelial cells but its distribution in these cells suggest that it is merely passing through rather than that it has been actively phagocytized as seen in the liver). In the late stage, the collargol is found in macrophages and free giant cells (Fig. 3), often collected in foci, in the pulp and in the germinal centers. The picture suggests that the collargol in single cells is increased in amount, while the number of such cells is decreased. Many of the collargol-containing cells have phagocytized nuclear débris. No collargol is seen in fixed endothelium.

Liver. In the animals killed early, the collargol is found to some extent in the fixed endothelium lining the sinusoids throughout the liver but especially either in giant cells or in macrophages collected in foci in the sinusoids (Fig. 3). About these foci, there are collargol-free macrophages and polymorphonuclear leucocytes. A rare mitotic figure is seen in collargol-filled macrophages. In the late animals, the collargol is in macrophages, usually collected in foci, and in giant cells in the sinusoids (Fig. 7). It is found also in macrophages in the lymphatics in the portal areas. Some of the collargol-containing macrophages have also phagocytized polymorphonuclear leucocytes and nuclear débris.

Kidney. In the early stages, there is some collargol both in free macrophages and in fixed endothelium in the glomeruli. It also occurs to a slight extent in the fixed endothelium of the small capillaries between the tubules and in macrophages in lymphatics. Late, there is only a small amount of collargol in this organ and that is in macrophages in the glomerular capillaries.

Adrenal. A small amount of collargol is found in macrophages in the capillaries.

Bone Marrow. In the early stages, the collargol is found in macrophages and in the fixed endothelium lining the blood spaces (Fig. 5). Late, it occurs exclusively in macrophages and giant cells, none being found in fixed endothelium (Fig. 8). These macrophages and giant cells are arranged in foci. One such giant cell contains besides the collargol five polymorphonuclear leucocytes and one lymphocyte.

Lymph Nodes. Considerable collargol is found in fixed endothelium and circulating macrophages in early stages (Fig. 4). In late stages no definite collargol is found.

Summary. In rabbits, collargol is taken up mainly in the liver, spleen, and bone marrow and to a slight extent in the lung, kidney and lymph nodes. In all organs, there seems to be a tendency for the collargol-containing macrophages to collect in foci as time goes on. The collargol gives rise to giant cell formation to a marked degree in the liver, spleen and bone marrow. In the rabbit as compared to the guinea-pig, the fixed endothelium, at least in the early stages, plays a greater part in taking up the collargol. That collargol does not interfere with phagocytosis of other material cannot be stated from our results, in spite of the fact that we often found cells containing both collargol and phagocytized cells and nuclear débris, since it is possible that such cells had phagocytized the other material before they took up the collargol. Collargol had no influence on the general condition of our rabbits as they remained perfectly well throughout and examination of their blood revealed nothing abnormal.

Collargol Rabbit Infected with Tuberculosis

As will be recalled, one of our collargol rabbits, R 3, after having received 37 cc. of a 1 per cent suspension of collargol, was given a heavy suspension of living human tubercle bacilli in its ear vein. This animal died thirty-nine days later. The histologic examination of its tissues are given below.

All the organs examined showed typical tuberculosis and in every organ tubercle bacilli were demonstrated by suitable staining methods.

Lung. Tuberculosis. The cells forming the tubercles are essentially free from collargol (Fig. 12). What little collargol is found occurs in macrophages collected in foci. The distribution of the collargol in these macrophages is striking and the description of it in this organ holds true of it in the other organs. The collargol in the macrophages is arranged in a spherical mass closely adjacent to the nucleus, often with a clear space in its center, in which can be distinguished the centrosomes. This arrangement corresponds closely to the "rosette" of the so-called monocyte as described by Sabin and her co-workers.

Spleen. Tuberculosis. Collargol is found in macrophages and in giant cells (Fig. 9). The cells forming the tubercles contain collargol.

Liver. Tuberculosis. The collargol occurs in macrophages in foci

(Fig. 10) and in giant cells, in the sinusoids and in the portal connective tissue. In the tubercles, a varying proportion of the cells contain collargol.

Kidney. Tuberculosis. No collargol is found in the tubercles or elsewhere.

Lymph Node. Tuberculosis. No collargol is found.

Bone Marrow. Tuberculosis. Collargol occurs in macrophages and in giant cells (Fig. 11). Two caseous tubercles are seen with free collargol in the necrotic tissue. There is little marrow activity and the majority of the cells seem to be lymphocytes and macrophages.

The interesting findings in this rabbit are two in number:

(1) The arrangement of the collargol in the macrophages. According to Cunningham, Sabin, *et al.*,[7] the monocyte phagocytizes material at the periphery of its cytoplasm, leaving its "rosette" free, whereas the clasmatocyte may arrange its phagocytized material in the "hof" next to the nucleus; following this criterion, these cells should be classified as clasmatocytes, since the collargol appears to be deposited in the very region in which Sabin located the hypertrophied rosette of the tuberculous monocyte. However, these same workers claim that the predominant cell in the formation of the tubercle is the monocyte. Now we found that many of the tubercles were formed in varying proportions by these collargol-containing macrophages which, as stated above, should be classified as "clasmatocytes." There are two possible explanations of these facts: either Sabin and her co-workers are mistaken in assigning the predominant rôle in tuberculosis to the monocyte, or collargol is arranged in monocytes in a manner different from any other material. It is interesting to note that Muller [8] had no hesitancy in classifying the cells containing collargol described in her experiments as clasmatocytes.

2. The fact that the proportion of cells forming the tubercles varied in their collargol content more or less directly with the amount of collargol in the organ. This would suggest that the cells forming the tubercle are derived purely locally.

Trypan Blue Rabbits

In order to study the distribution of trypan blue in rabbits, two additional animals were given daily injections of a filtered 1 per cent saline suspension of the dye over a period of seventeen days. The dye injected totaled 69 cc. Both animals were bled and then killed on the day following the last injection. The serums were deeply stained and the dye content was roughly estimated colorimetrically at 0.025 gm. per 100 cc. of blood. A similar titration of the trypan blue rabbit used in the above experiments showed 0.004 gm. per 100 cc. of blood. Complement titrations in these animals showed no variation from controls. Tissues examined histologically (frozen sections and rapidly prepared, alcohol-formalin fixed celloidin sections) showed trypan blue distributed as follows:

Lung. In one rabbit, the dye is in cells in the capillaries scattered throughout the lung. In the other rabbit that has an incidental bronchopneumonia, the trypan blue occurs in cells in foci about the larger blood vessels and bronchi; it also is found in cells in the exudate in the alveoli and bronchi.

Spleen. By far the major portion of the trypan blue is in macrophages in the pulp; a small amount is found in the fixed endothelium.

Liver. The dye occurs both in the fixed endothelial cells lining the sinusoids and to some extent in the parenchymatous cells.

Kidney. The trypan blue occurs exclusively in the convoluted tubules. None is found in the glomeruli or endothelium elsewhere.

Lymph Node. Both the macrophages in the sinuses and the fixed endothelial cells lining the sinuses contain trypan blue.

Bone Marrow. The trypan blue is found in both the fixed endothelial cells lining the blood spaces and in macrophages scattered through the hematopoietic tissue.

Summary and Conclusions

1. Collargol injected repeatedly intravenously in guinea-pigs produced the following effects on immunologic responses:
   (1) depression of precipitin formation;
   (2) depression or stimulation of agglutinin production; and
   (3) protection against anaphylactic shock.
2. Rabbits treated in a similar manner showed the following:
   (1) depression of precipitin production;

(2) slight or no stimulation of agglutinin or hemolysin production; and

(3) no protection against anaphylactic shock.

3. Histologic studies indicate that blockade of fixed endothelium is not obtained with either collargol or trypan blue except in the liver and to slight extent in lymph nodes and bone marrow. Once attained, continued injection is necessary to keep the blockade advanced over the segregation of blocked cells, and to maintain block a considerable amount of circulating colloidal material is needed. In view of this fact it is difficult to assert categorically that resultant alterations in antibody production are due to endothelial blockade.

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DESCRIPTION OF PLATES

Plate 75

Fig. 1. Spleen, late guinea-pig; sclerosis. × 250.
Fig. 2. Spleen, early rabbit. × 250.
Fig. 3. Liver, early rabbit; granules in liver cells not collargol. × 250.
Fig. 4. Lymph node, early rabbit. × 1000.

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Fig. 5. Bone marrow, early rabbit. × 500.
Fig. 6. Collargol-filled giant cell, spleen, late rabbit. × 750.
Fig. 7. Liver, late rabbit; granules in liver cells not collargol. × 250.
Fig. 8. Bone marrow, late rabbit; these marrows were not uniformly aplastic. × 500.

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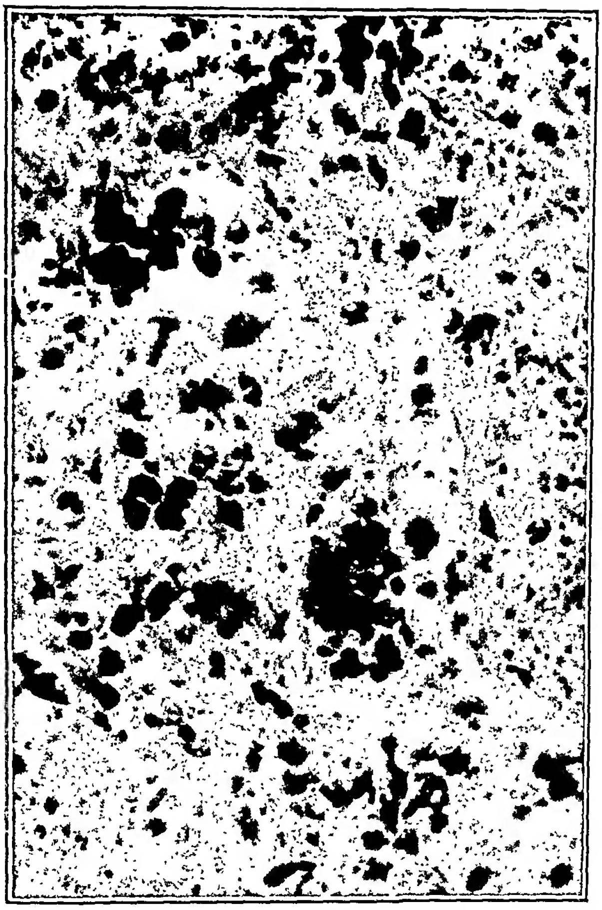
Fig. 9. Spleen, tuberculous rabbit. × 750.
Fig. 10. Liver, tuberculous rabbit. × 750.
Fig. 11. Bone marrow, tuberculous rabbit. ×750.
Fig. 12. Lung, tuberculous rabbit. × 750.



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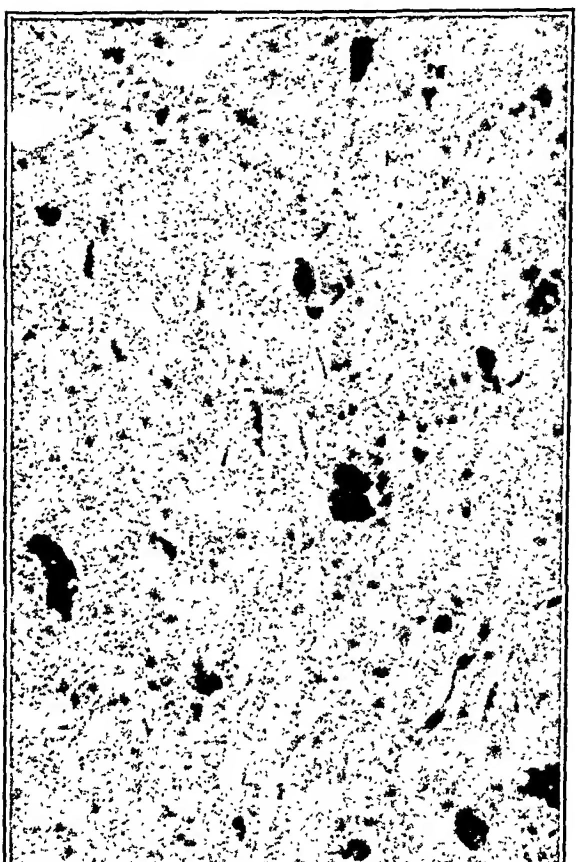

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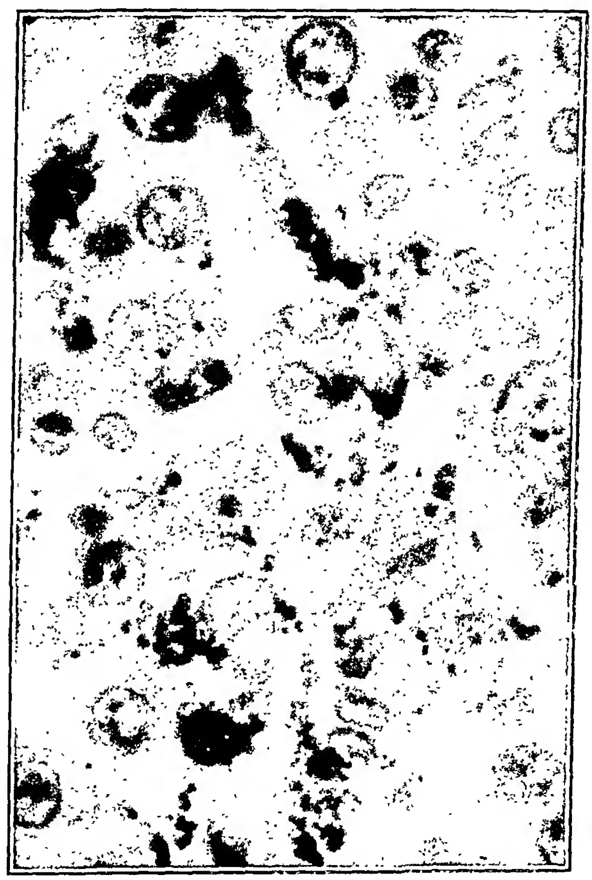

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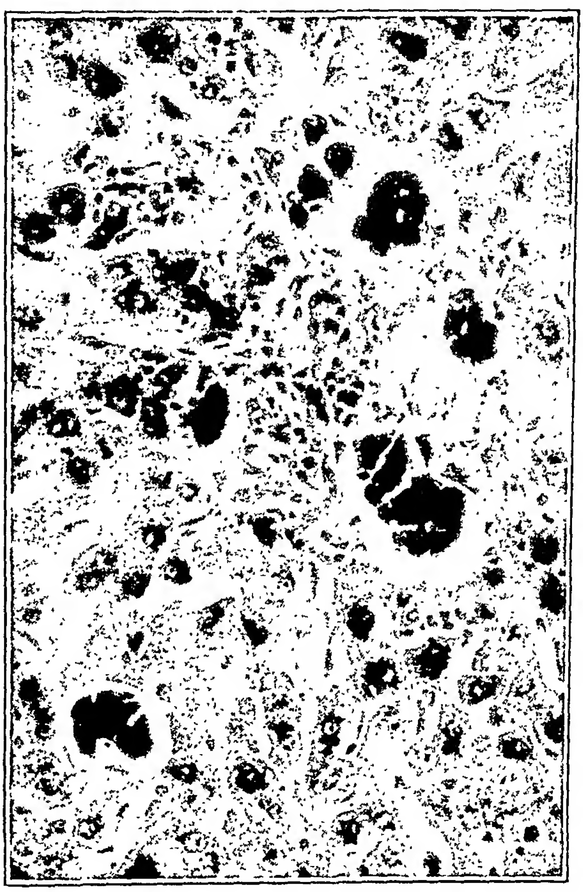

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Stewart and Parker

So-called "Endothelial Blockade"

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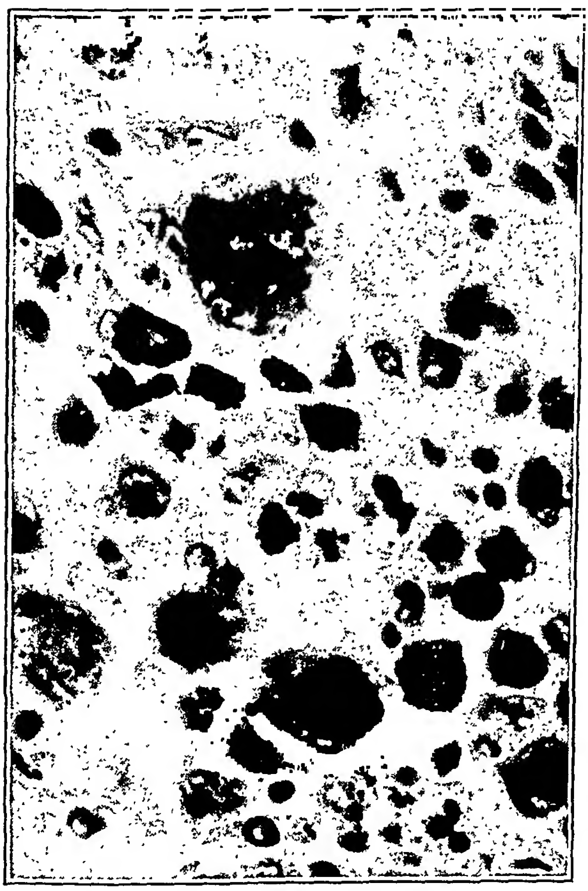

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12

CASES OF RENAL INFECTION IN PULMONARY TUBERCULOSIS

EVIDENCE OF HEALED TUBERCULOUS LESIONS *

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Renal tuberculosis as seen in clinical practice comprises, as stated by Caulk,[1] 30 per cent of all surgical lesions of the kidney. These cases are usually diagnosed at a date when there is no apparent clinical manifestation of tuberculous lesions in any other part of the body and hence are looked upon as primary lesions. The extensive destruction of kidney tissue as found in such cases has led to the belief that tuberculosis of the kidney is a progressive destructive lesion which does not heal. Because of this interpretation it is now a common belief that whenever tubercle bacilli are found in one ureteral urine and not in the other, the kidney from which the bacilli are excreted should be removed regardless of the size of the lesion. The truth or falsity of this belief, that renal tuberculosis does not heal, is the main consideration of this paper.

Renal tuberculosis with cavitation, that is renal phthisis, would appear to correspond to the stage in pulmonary tuberculosis where cavitation is present. They are both advanced stages of the disease. Since it is a well established fact that all cases of tuberculous infection of the lung do not go on to cavitation, it would seem quite probable that the same fact would apply to tuberculous infection of the kidney. If the pathologic study of pulmonary tuberculosis were limited to cases with cavitation it would not give a true conception of the disease in its entirety as it occurs in the lung. It would seem plausible that this might also be the case in tuberculous infection of the kidney.

With the above consideration in mind, it was decided to study the kidneys from patients dying of pulmonary tuberculosis. The cases chosen gave no clinical symptoms of renal involvement. No case of renal phthisis was included. Thirty cases were selected all of which showed active tuberculous lesions of the lung with caseation. The

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immediate cause of death was advanced pulmonary tuberculosis in twenty-seven cases, tuberculous meningitis secondary to pulmonary tuberculosis in two cases and streptococcal abscesses of the brain with advanced pulmonary tuberculosis in one case. The ages range from 18 months to 70 years.

Both kidneys were examined in fourteen cases. In sixteen cases only one kidney was available. In all, forty-four kidneys have been examined.

The technic of gross examination used was as follows. The kidneys were split open lengthwise and fixed *in toto* for forty-eight to ninety-six hours in 10 per cent formalin. They were then separated into two halves and each half was cut into strips from two to three millimeters in thickness. Portions of the kidney tissue which appeared abnormal were cut out for microscopic study. In those cases where definite tuberculous lesions were numerous, the smaller lesions only were saved. All areas where there was a depression in the kidney surface were also removed for histologic examination. With this technic it was much easier to be certain of small lesions in the kidney tissue than in fresh unfixed organs. The technic is admittedly faulty in that without doubt many small lesions were overlooked. But the labor involved in proper histologic preparations of entire kidneys necessitated the removal of small blocks of tissue for microscopic study.

The blocks of tissue were embedded in paraffin in the usual manner. The tissue was then serially sectioned and mounted on glass slides. Every third slide was stained for tubercle bacilli by the use of Verhoeff's carbol-fuchsin. The sections were first overstained with hematoxylin and then placed in cold carbol-fuchsin over night. One per cent hydrochloric acid in 80 per cent alcohol was used as the discharging fluid, the sections being treated for five to ten minutes. The remainder of the sections were stained with hematoxylin and eosin.

In this study approximately 100,000 sections have been carefully examined. Serial sections were found invaluable as entire lesions could be followed through. In the case of scars it was thus possible to determine whether the reparative process simply surrounded a lesion incompletely healed or whether the lesion was completely healed. As high as fifty sections in some of the scars were carefully examined for the presence of tubercle bacilli. By this method it was

also possible to demonstrate tubercle bacilli in lesions where single sections would have yielded negative results. In some lesions the bacilli were so scarce that but one bacillus could be found in ten sections. In other lesions a single section would show hundreds of the organisms. With this technic one was able to determine whether the lesion was entirely cortical, entirely medullary or cortico-medullary. It was possible also to find many microscopic lesions that would have escaped detection by any other technic.

In all, 367 definite tuberculous lesions were studied. These lesions varied from small tuberculous abscesses to lesions in which the only evidence of their tuberculous nature was revealed by the presence of an occasional giant cell or of a microscopic mononuclear tubercle. Tubercle bacilli were found in 265 of these lesions. Many of the small lesions were not stained for tubercle bacilli. Others were stained but no tubercle bacilli were found and in these cases the only evidence that the process was tuberculous in nature was the presence of one or more giant cells. There was great variation in the number of tubercle bacilli in the different cases. In one case fifty-four separate lesions were studied and tubercle bacilli were numerous in every lesion. In another case twenty-nine separate lesions were studied and tubercle bacilli were found, after prolonged search, in but one lesion.

The distribution of the lesions within the kidney tissue was as follows. Cortical lesions numbered 277 or a fraction over 75 per cent; medullary lesions were present forty times or about 11 per cent; and cortico-medullary lesions numbered 50 or about 13 per cent.

A study of the point of origin of the lesions proved of interest. For this study the smaller or microscopic lesions were relied upon, as the larger lesions involved too much tissue to make it possible to determine with assurance the exact point of origin. The smaller lesions were of two main types. The most common of these types was of vascular origin within the capillary tuft of a glomerulus, within a capillary between the convoluted tubules or within a capillary between the collecting tubules in the pyramids. Of these points of origin the most common was within a glomerulus and the least common in the pyramid. In the early glomerular lesion several instances were found in which one half of the glomerulus was involved while the remainder was normal.

The second type of small lesion had its origin within the lumen of

tubules. These lesions were found with about equal frequency in the lowest point of the loop of Henle and in the collecting tubules in the pyramid. They always subtended an ulcerating tuberculous lesion of some portion of the kidney substance. It appears that this type of lesion is always secondary to a vascular lesion which has developed to a point where destruction of tissue has supervened and tubercle bacilli have been discharged into the lumen of a tubule. In some instances the primary vascular lesion was a small tuberculous abscess involving but one glomerulus.

The points of prime importance in this study were the search for scars in the kidney tissue and the study of them. At the outset the difficulty of the interpretation of scars in the kidney was realized. Scars as the end result of infarction are so typical that their interpretation affords little difficulty. Scars occurring in kidneys where there was evidence of atherosclerosis were disregarded in the study. The healed lesion of infections other than tuberculosis would appear to be impossible to separate from healed tuberculous lesions in which caseation had been absent or at most was scanty. In other words, in a considerable portion of healed tuberculous lesions one could hardly expect to find anything which would label it as tuberculous in nature. This certainly is true in other organs of the body. Sclerosed glomeruli even in young individuals where there was no evidence of atherosclerosis were disregarded in this study, although it was felt that in all probability at least some of them represented the healed stage of a tuberculous process within the glomerulus.

Of the thirty cases studied, two gave no evidence of tuberculous lesions or scars. In twenty-two cases, definite tuberculous lesions were found and of these cases fourteen showed scars also. The remaining six cases showed scars only. Of the twenty cases which showed scars, three showed definite atherosclerotic lesions. Two of these sclerotic cases had definite tuberculous lesions and the third did not. The seventeen cases which did not have atherosclerotic lesions were also free from infarction except in connection with some of the larger tuberculous lesions. Not all of the scars were tabulated, but the following was the distribution of 100 of these lesions: cortical, 80; medullary, 14; and cortico-medullary, 6. From this it will be seen that the scars were distributed in the different renal regions in approximately the same proportion as were the active lesions.

The size and histologic picture of these scars varied considerably. Most of the scars were microscopic in size. Thirty-one scars of macroscopic size were found and the largest of these measured 3 mm. in diameter. All of the lesions showed destruction of kidney tissue, which varied from very slight to a rather large area of destruction, with a replacement of the area by connective tissue. In some of the larger lesions lymphocytic infiltration was marked but this was not constant, for many of the lesions showed no lymphocytic infiltration except perhaps an occasional lymphocyte in the tissue around the periphery. The density of the connective tissue varied greatly, being very compact in a few of the scars and of a rather loose texture in the majority of the lesions.

In all of the larger lesions some normal kidney tissue was present within the scarred area. If the lesion were cortical, normal glomeruli and convoluted tubules were present, while in the medullary region normal collecting tubules were present. These normal structures were always much fewer in number in these areas than in the surrounding normal tissue. At the periphery of the lesion it was common to find dilated tubules filled with hyaline material. This condition was interpreted as being due either to pressure upon a tubule as it passed through the scarred area or to the destruction of the portion involved in the scarred area with the peripheral portion remaining functional. In some of the larger cortical lesions a rare sclerosed glomerulus was found. Four scars contained small irregular particles which resembled bits of old caseous material.

One case in this series will serve to illustrate a condition which is probably quite common among tuberculous individuals. The patient was a man of about 35 years who entered the sanatorium at Mt. McGregor, New York, eight years before his death, as a case of far advanced pulmonary tuberculosis. For two years he underwent rigid sanatorium treatment. At the end of this time he was sufficiently improved to run the print shop at the sanatorium. This work he continued uninterruptedly until two weeks before his death. Monthly examinations were made during these six years and there was no evidence of a recrudescence of the pulmonary lesion, though his sputum remained positive. He never had any clinical symptoms pointing toward renal involvment. About two weeks before his death he developed a hemiplegia following a rather severe bronchitis.

Necropsy of the above case showed death to be due to two streptococcal brain abscesses. One lung showed an old fibroid phthisis and the other multiple old tuberculous lesions. There were many old tuberculous lesions in the spleen and liver.

Both kidneys showed tuberculous lesions. Six thousand sections from these kidneys were examined. There were 33 tuberculous lesions of which 25 were cortical, 5 were cortico-medullary and 3 were medullary. Tubercle bacilli were extremely hard to find and were demonstrated in but nine lesions. Only six lesions showed areas of caseation. In many of the lesions the only evidence suggesting tuberculosis was the presence of one or more giant cells. Besides these lesions there were numerous scars such as have been described above. These scars were present in the cortex and in the pyramids. Two lesions which in gross appeared to be caseous areas proved on section to be old caseous material, containing numerous cholesterin crystals, with a fibrous wall infiltrated with lymphocytes and an occasional mononuclear leucocyte completely surrounding it. Over 200 sections in these two lesions were carefully searched for tubercle bacilli. Two bacilli were found in one lesion and none was found in the other. These lesions at a previous date were undoubtedly active, ulcerating, caseating lesions. When examined, while not completely healed, they were so thoroughly walled off as to be innocuous to the individual. It would seem highly improbable that tuberculous kidneys such as these would excrete tubercle bacilli in the urine at this stage.

As stated above, both kidneys were examined in fourteen cases. Two of these cases were entirely negative as far as my examination went. One case showed only scars in both kidneys. The remaining eleven cases had tuberculous lesions in both kidneys. In several cases the lesions were larger in one kidney than in the other. The reason for this difference is not certain, although the chance of bacillary dosage would appear the most logical explanation.

Small benign tumors were encountered in six of the thirty cases. These tumors were multiple. In three cases papillary adenomas of the cortex were present. Two cases showed fibromas of the medullary region. One case had both types of tumor present. Microscopically, neither type of tumor could be confused with scars or active tuberculous lesions, as they were encapsulated and had definite tumor architecture.

As a control to the above series of tuberculous kidneys, the kidneys from twenty-two necropsies on non-tuberculous individuals were examined by the same technic. Only sufficient sections were examined to ascertain the nature of the pathologic lesions found. It was not deemed necessary to follow the lesion through in serial section. The age range of the cases was from 6 months to 76 years. The list of diseases is as follows: bronchopneumonia, 3; pernicious anemia, 2; ascending bilateral pyelonephritis, 3; endocarditis, 3; cardio-renal, 3; malignancy, 4; chronic glomerulonephritis, 1; gummas of liver with ruptured esophageal varix, 1; and *Staphylococcus aureus* septicemia, 2.

The lesions observed in the kidneys of these cases were as follows: atherosclerotic scars, 7; infarcts (cases of endocarditis), 2; chronic glomerulonephritis, 1; acute infectious nephritis with abscess formation, 5; small cortical scars, 2 (both were cases of endocarditis); fibroma (medullary), 1; papillary adenoma (cortical), 2; and negative, 8.

The kidneys showing infectious lesions and scars were more thoroughly studied than the remaining cases. The cases of *Staphylococcus aureus* infection were in young individuals and the distribution of the lesions in the kidney tissue was very similar to the distribution in tuberculous infection. Cortical lesions were by far the most common, but cortico-medullary and medullary lesions were also present. The lesions were bilateral in both instances.

The examples of bilateral pyelonephritis occurred in adult males and were the result of obstruction in the lower genito-urinary tract. One of these cases showed rather extensive atherosclerotic scars. A second had slight diffuse scarring but no healed lesions similar to those seen in the tuberculous cases and the third was devoid of scars.

The two kidneys which showed cortical scars were in young adults with endocarditis. There was no evidence of atherosclerosis. All of the scars observed were cortical and appeared to be healed infectious lesions. These lesions were not numerous. They were indistinguishable from many of the scars found in the tuberculous kidneys.

Discussion

In order to interpret the pathologic picture presented in any tuberculous process it is essential to have in mind the pathogenesis of the tuberculous lesion. For a full consideration of the cytologic reaction

and its meaning in tuberculosis the reader is referred to two articles now in process of publication. These articles will appear in the American Journal of Pathology and they express my interpretation of the tuberculous process. A very brief résumé of this interpretation will be given here to aid in clarifying the opinions given below.

The first and typical reaction to the tubercle bacillus is the "epithelioid" or mononuclear tubercle. If the individual has high resistance, caseation does not ensue and the hyperplastic type of tuberculosis is produced. In such cases, if the tubercle bacilli are destroyed, as undoubtedly they often are, the end result is a small scar.

If the individual is unable to cope with the infection, polymorphonuclear leucocytes are attracted to the injured tissue and an abscess is formed. In case this area is so situated that the necrosing tissue can be discharged to the outside, ulceration or cavitation occurs. It is at this stage of the process that tubercle bacilli occur in the sputum or in the urine. In case this discharge cannot occur, caseation supervenes through the death and disintegration of all cells within the abscessed area. Subsequent to caseation the polymorphonuclear leucocytes do not appear to be further attracted to the lesion.

Following caseation, that is in the reparative stage of the disease, the mononuclear leucocytes and lymphocytes are attracted in large numbers. It is in this stage of the process that giant cells appear, so that whenever giant cells occur in a tuberculous lesion it is definite evidence of a reparative reaction on the part of the host. As a reparative process goes on toward completion the need for mononuclear leucocytes and lymphocytes becomes less and less until the end product is a scar with nothing pathognomonic of the etiologic factor which was the responsible agent in the infection.

If the above interpretation of the tuberculous process from its inception to the healed stage is, in the main, correct, then the interpretation of the tuberculous lesions as found in the kidney can be set forth with a fair degree of accuracy. From this study it appears that the tuberculous lesion in the kidney does not differ from that in other organs and tissues of the body, providing the differences in histologic and anatomic structure are borne in mind. As far as can be determined, the same types of cells participate in the defensive and reparative processes of the tuberculous lesion in all tissues and organs.

It was not uncommon in this study to find a great variety of tuberculous lesions in a single kidney. Mononuclear tubercles, tuberculous abscesses, areas of caseation, scarred areas infiltrated with lymphocytes and with one to many giant cells present, and scars devoid of lymphocytic or mononuclear leucocytic infiltration have all been observed in one organ. From this is would appear that the individual had had, at intervals, showers of tubercle bacilli in the blood stream and these showers have been followed by the development of tuberculous lesions in the kidney. The pathologic processes found in such organs represent, then, lesions of different age and severity, and the scars represent the healed stage in an area where the tubercle bacilli have been successfully overcome. The fact that out of twenty-two cases with definite tuberculous lesions, twelve with no evidence of atherosclerosis showed scars, whereas out of twenty non-tuberculous cases, in only two with no evidence of atherosclerosis were scars found, leads one to the logical conclusion that at least a portion of these scars, and in all probability a goodly portion, represent healed tuberculous lesions.

The cases cited above did not show completely healed renal tuberculosis. At least three of the cases would have gone on to extensive destruction of kidney tissue, if one may forecast this condition from the numbers of tubercle bacilli present and the severity of the inflammatory process. On the other hand, five cases out of thirty studied showed scars only. These cases showed no evidence of atherosclerosis. If one grants that at least a portion of these scars represent healed tuberculous lesions, then there is definite evidence that, under favorable circumstances, renal tuberculosis can heal completely.

A fact of some surprise in this study was that none of the cases presented clinical manifestations pointing toward renal involvement. A study of the urine for tubercle bacilli was not made. Judging from the presence of inflammatory exudate and tubercle bacilli in the lumen of tubules, six of the cases or 20 per cent should have had bacilluria. Brown [2] reported 10 per cent positive urines in 104 cases and Hobbs [3] reported 6 per cent in 100 cases where there were no clinical manifestations of renal involvement. So it is apparent that renal tuberculosis with bacilluria can exist without causing clinical manifestations. The authors here quoted, and others, believe that bacilluria is at times encountered in the absence of kidney lesions.

My belief is that "excretory bacilluria" does not exist without ulcerative tuberculous lesions in the kidney. That these lesions are often microscopic and are many times overlooked is in all probability the reason for the belief in "excretory bacilluria." Such lesions may involve but a part of one glomerulus.

If one may judge by the absence of inflammatory exudate and bacilli in the lumen of tubules at least one-half of the cases in this series would not have shown tubercle bacilli in the urine. From this it would seem that renal tuberculosis can exist without bacilluria being present.

The common occurrence of tuberculous lesions in the kidney in cases of pulmonary tuberculosis was unexpected. This report shows a very high percentage. If a much larger number had been examined the percentage might have been lower. A probable explanation of the findings is that the examination of the kidneys have been much more thorough and that serial sections have revealed many lesions unsuspected on gross examination. If the findings in this study represent the true facts, it is apparent that every case of progressive pulmonary tuberculosis is a potential candidate for renal infection. It would also seem that cases of renal tuberculosis are secondary to some other tuberculous focus, usually pulmonary, in the body and that the infection is hematogenous. That the infection is hematogenous is indicated by the preponderance of cortical lesions.

Caulk [1] states that there is no authentic case on record of healed tuberculosis of the kidney. I have not been able to find such a case recorded. Hobbs [3] states in his article that he found an occasional scar and gives an illustration of the lesion. The illustration appears more like the fibromas I have encountered than like the scars I have described above. It would appear that the reason for no recorded case of healed renal tuberculosis is that the majority of cases studied have been of renal phthisis and that where tuberculosis of the kidney has been observed in the routine of a necropsy its occurrence has been automatically recorded without a thorough systematic study of the kidney with a view to determine the possibility of healed lesions in the same kidney. Renal phthisis does not afford suitable material for the study of healing of the tuberculous process, as it is an advanced progressive lesion. Healing of a cavitated kidney probably

does not occur. There is reason to believe, however, that under suitable circumstances such lesions may be clinically arrested.

It is now known that tuberculous lesions of the lung, of the intestine and of other tissues do heal. With the evidence given above regarding scars in the kidney in cases of tuberculosis, it would seem illogical to maintain the attitude that renal tuberculosis never heals.

The purpose of this paper is to present as completely and as concisely as possible the pathologic side of renal tuberculosis. The clinical side is another study but it would seem plausible that the more nearly the pathology is understood, the more sane will be the course of clinical treatment advised. The following pathologic facts are emphasized. The presence of tubercle bacilli in ureteral urine establishes the diagnosis of renal tuberculosis but not of renal phthisis. The absence of tubercle bacilli in the urine does not rule out tuberculosis of the kidney. Renal tuberculosis is of hematogenous origin and, as far as this study goes, when it occurs it is always bilateral. Tuberculous lesions of the kidney heal. Renal tuberculosis can exist without clinical manifestations.

With the above discussion in mind it would seem advisable to establish the following facts before nephrectomy for renal tuberculosis is undertaken: (1) evidence of considerable destruction and cavitation of the kidney; (2) the presence of tubercle bacilli in the urine on several examinations; (3) the absence of tubercle bacilli in the urine from the opposite kidney on several examinations; and (4), the failure of treatment on the same basis as for pulmonary tuberculosis to arrest the condition.

I have not felt it advisable to quote extensively from the literature on renal tuberculosis in this article. For the more important articles on the subject the reader is referred to the bibliography in a previous article by Dr. Sasano and myself.[4]

Conclusions

1. Renal tuberculosis is common in advanced pulmonary tuberculosis, twenty-two out of thirty cases.

2. Renal tuberculosis is hematogenous in origin, 75 per cent of the lesions being cortical.

3. Bilateral infection was the rule in every case in this series in which both kidneys were examined and tuberculous lesions were present.

4. Tuberculous lesions of the kidney heal. Scars were present in seventeen out of thirty cases. Twelve of these cases also had tuberculous lesions.

5. Serial sections are invaluable in such a study.

REFERENCES

1. Caulk, J. R. *J. Urol.*, 1921, vi, 97.
2. Brown, L. *J. A. M. A.*, 1915, lxiv, 886.
3. Hobbs, F. B. *Tubercle*, 1923, v, 57 and 105.
4. Medlar, E. M., and Sasano, K. T. *Am. Rev. Tuber.*, 1924, x, 370.

DESCRIPTION OF PLATES

Plate 78

Fig. 1. A tubercle in a glomerulus. The lower part of the glomerulus is not involved. The tubercle shows evidence of injury to the tissue but no caseation. The capsule and pericapsular tissue is involved to the left. Sclerosed glomeruli showing only partial involvement of the capillary tuft such as this have been found. × 350.

Fig. 2. A small tuberculous abscess between the tubules in the cortex. The inflammatory exudate consists largely of polymorphonuclear leucocytes. The tubules to the left contain inflammatory exudate and tubercle bacilli. There is no evidence of caseation. The lesion is microscopic in size. × 350.

Plate 79

Fig. 3. A tubercle in a glomerulus undergoing caseation at its periphery. This is in reality a small tuberculous abscess. Note the normal portion of the glomerulus above. × 350.

Fig. 4. A caseous lesion undoubtedly arising in the glomerulus in the center of the field. The dark portion is closely packed with "nuclear dust." This is a later stage of a lesion like Fig. 3. × 350.

Plate 80

Fig. 5. A microscopic mononuclear tubercle in the tissue to the right of the glomerulus. × 350.

Fig. 6. A scar in a position similar to the tubercle in Fig. 5. Such scars were quite frequently found and represent what I believe to be healed tubercles which have not gone on to caseation. Note the fibrous thickening of the capsule of Bowman. × 350.

Fig. 7. A giant cell tubercle. This was the only evidence of tuberculosis in a series of 300 sections from a block of kidney tissue. Other larger tuberculous lesions were found in this same kidney. This giant cell extended through 20 sections 10 microns thick. It represents a tubercle which has undergone necrosis or caseation, probably the latter, and which is now undergoing repair. × 350.

PLATE 81

FIG. 8. A large cortical scar of macroscopic size with normal glomeruli and tubules in it. There is marked fibrosis and some lymphocytic infiltration. No evidence that this lesion was tuberculous was found but there were low grade tuberculous lesions in the same kidney. Note the dilated tubules above. This is a healed infectious lesion and the probability is in favor of it being a healed tuberculous lesion. × 100.

FIG. 9. A higher power of Fig. 8, showing a normal tubule, scar tissue and some lymphocytic infiltration. × 350.

PLATE 82

FIG. 10. A medullary scar of macroscopic size. In other sections this scar contained two dilated collecting tubules. × 100.

FIG. 11. A tuberculous lesion. The only evidence is three giant cells and a small mononuclear tubercle above. After prolonged search one tubercle bacillus was found. For all practical purposes this lesion is healed though pathologically it is not healed. × 200.

FIG. 12. A tuberculous lesion. The only evidence in over 100 sections was these two giant cells. No tubercle bacilli were found. × 350.

PLATE 83

FIG. 13. A large cortical scar from a tuberculous case. No active tuberculous lesions were found in this case. × 100.

FIG. 14. An old caseous area walled off by fibrous tissue. This is one of the lesions mentioned in the text. No tubercle bacilli were found after very careful search. × 100.

PLATE 84

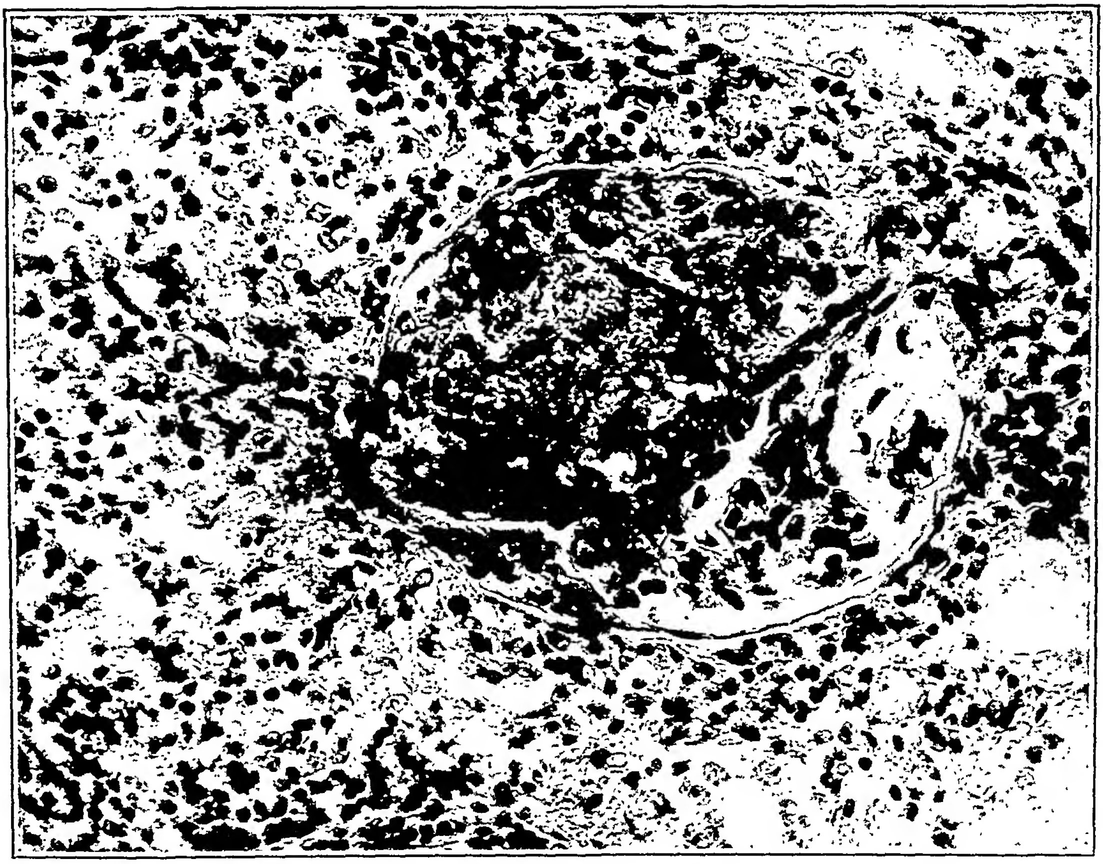
FIG. 15. A large cortical scar. The only evidence of its having been tuberculous is a giant cell at the left. × 200.

FIG. 16. A large medullary scar of macroscopic size. Note the normal tubules within the scarred area. Some of the tubules are considerably dilated. There are also small irregular hyaline masses in the tissue which I take to be bits of old caseous material. Four such scars were found in three cases of this series. × 200.

PLATE 85

FIG. 17. A medullary fibroma. Compare the architecture of the tumor with that of the scars in Fig. 9, 10, 12, 16 and 18. × 100.

FIG. 18. Dense fibrous scar. This represents a healed caseated tuberculous lesion. The sclerosed glomeruli shown above are typical of such lesions commonly seen in tuberculous kidneys which show no evidence of atherosclerosis. × 200.



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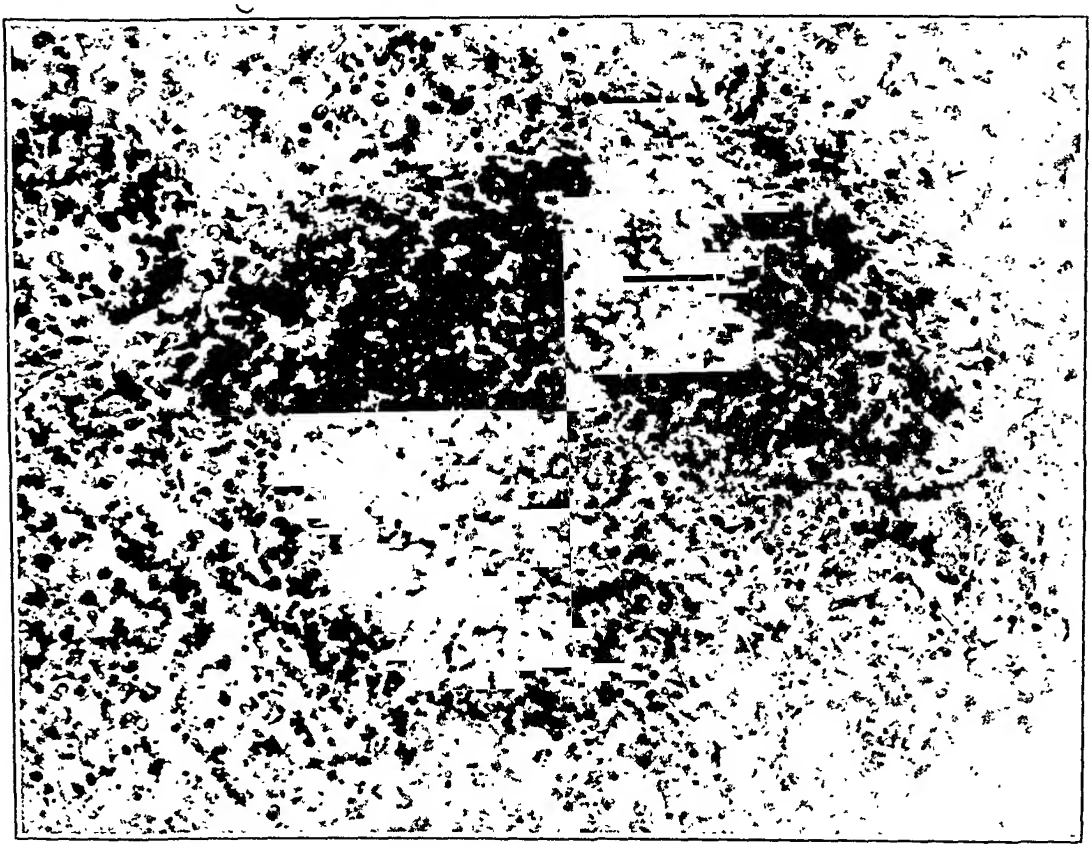

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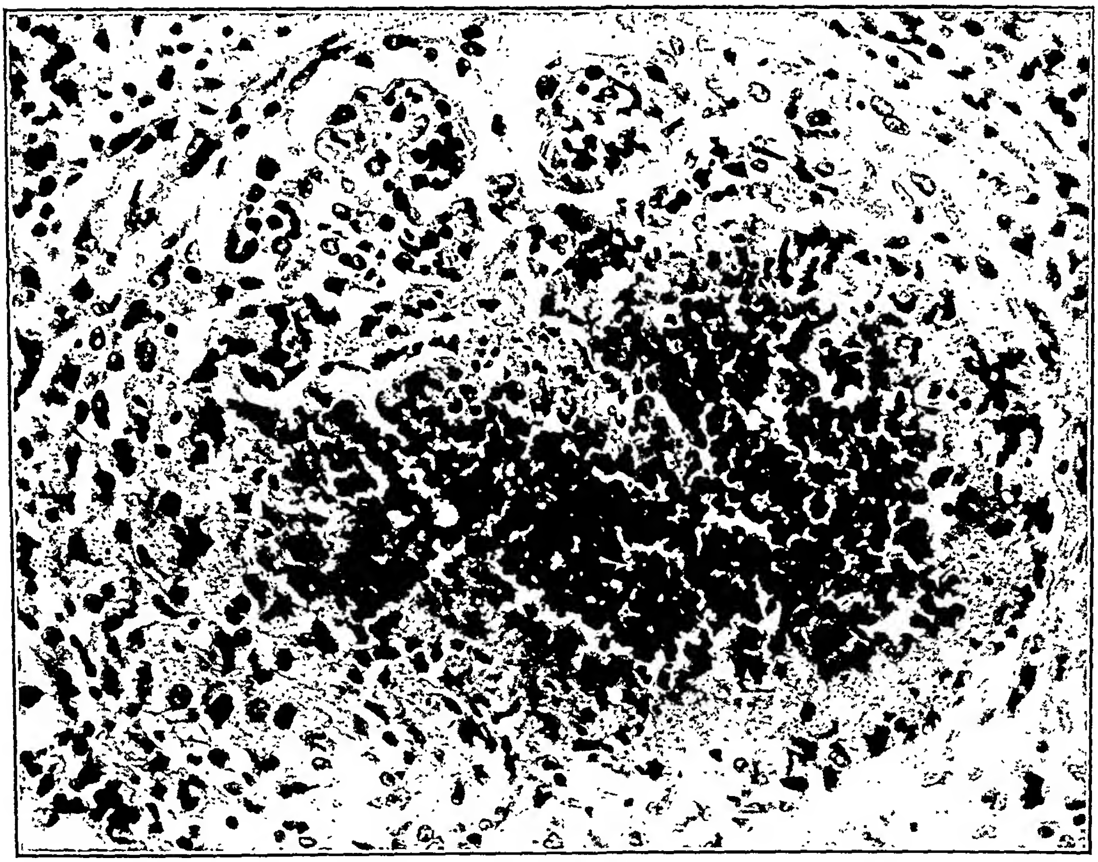

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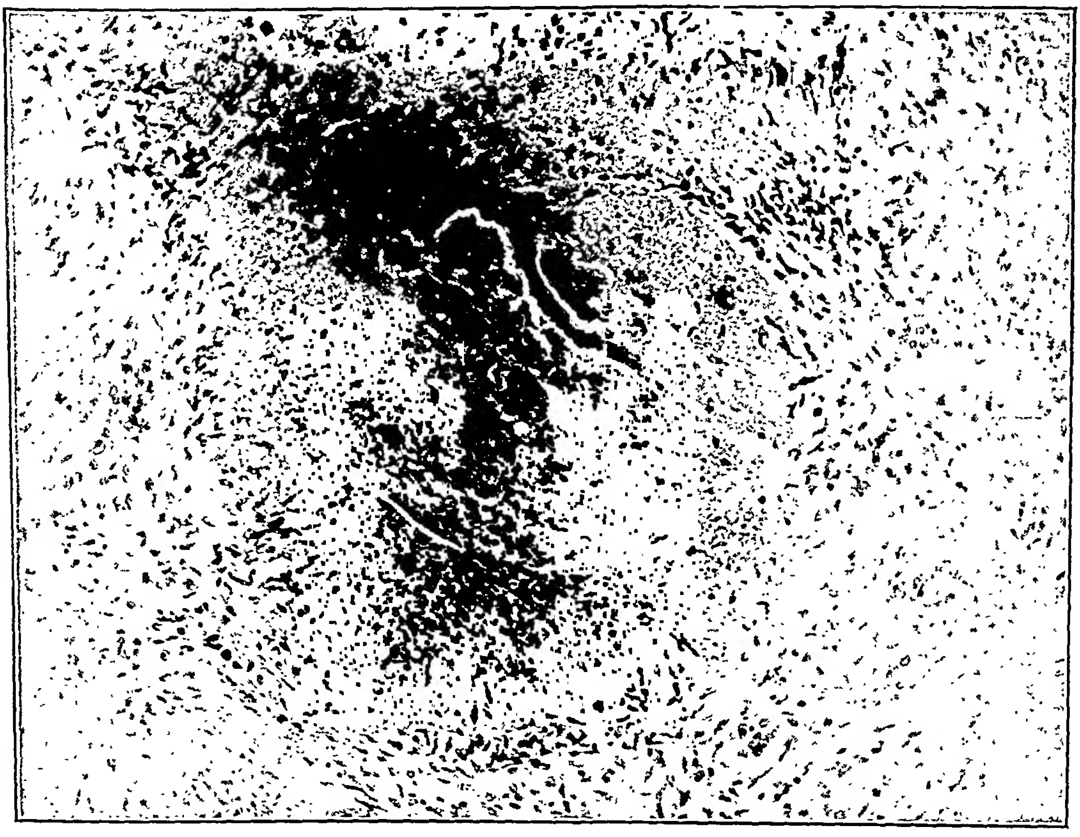

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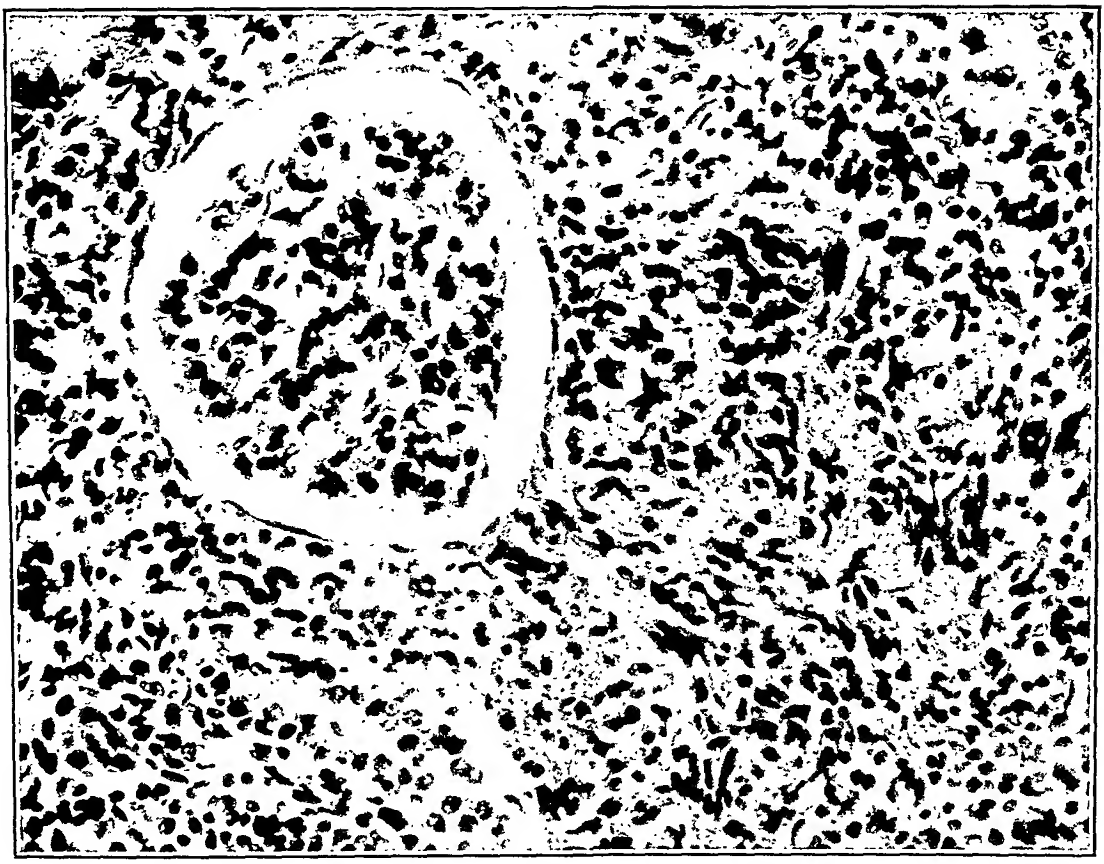

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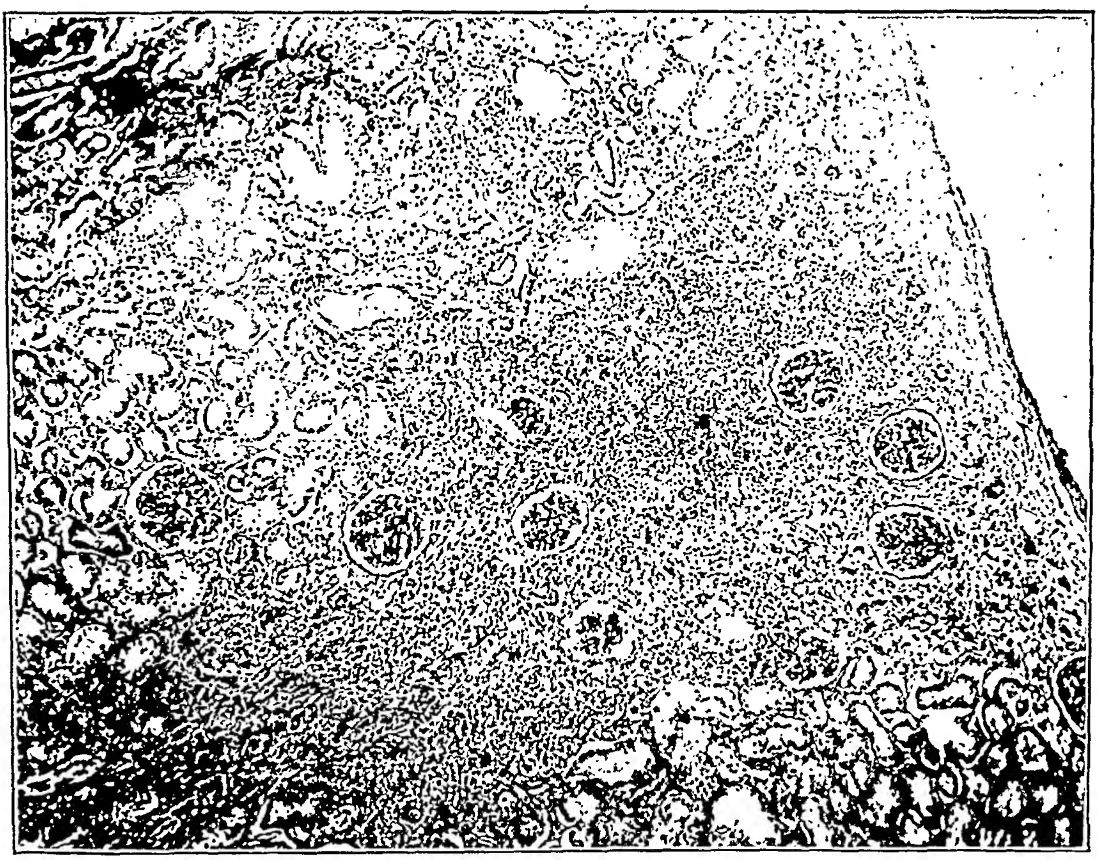
Medlar

Renal Infection in Pulmonary Tuberculosis



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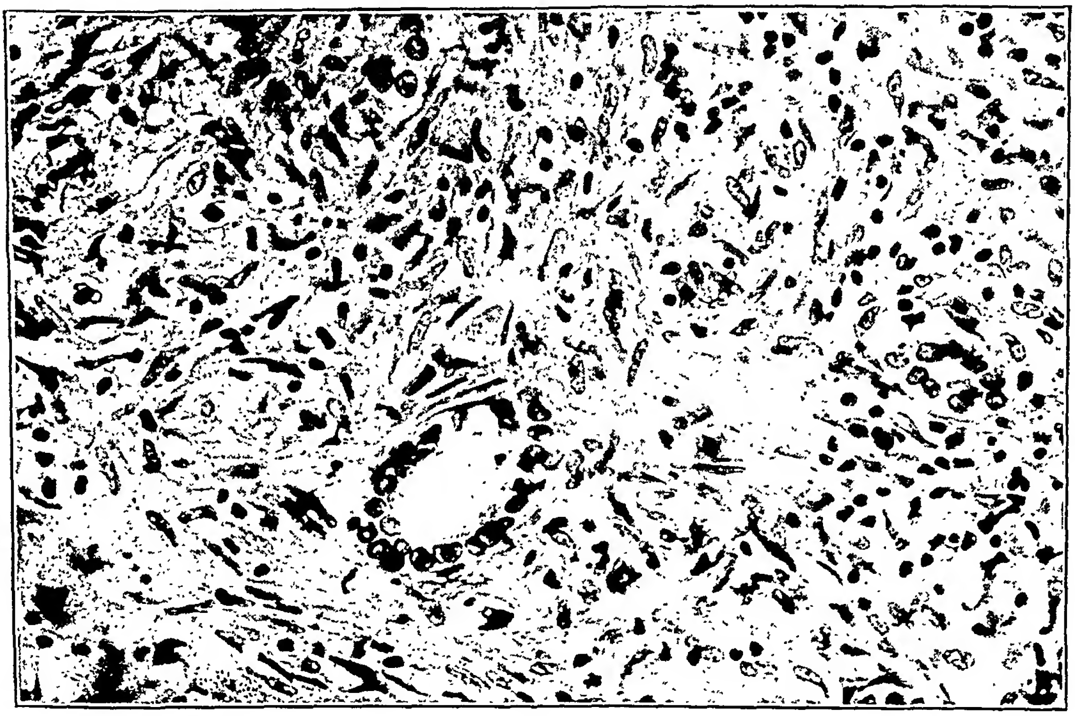

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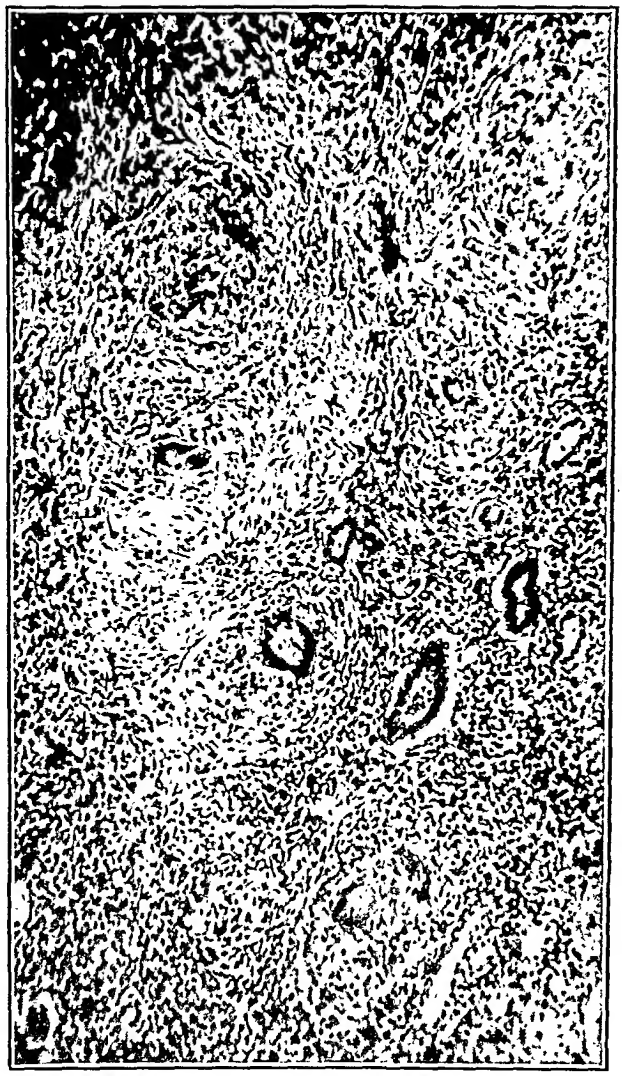

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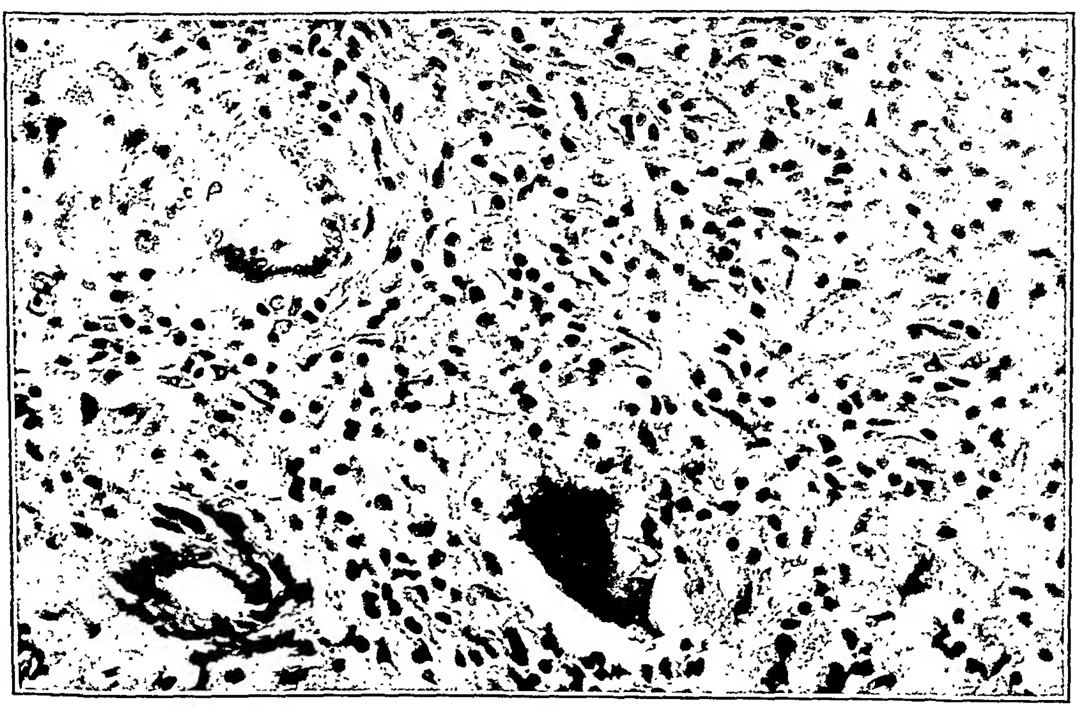

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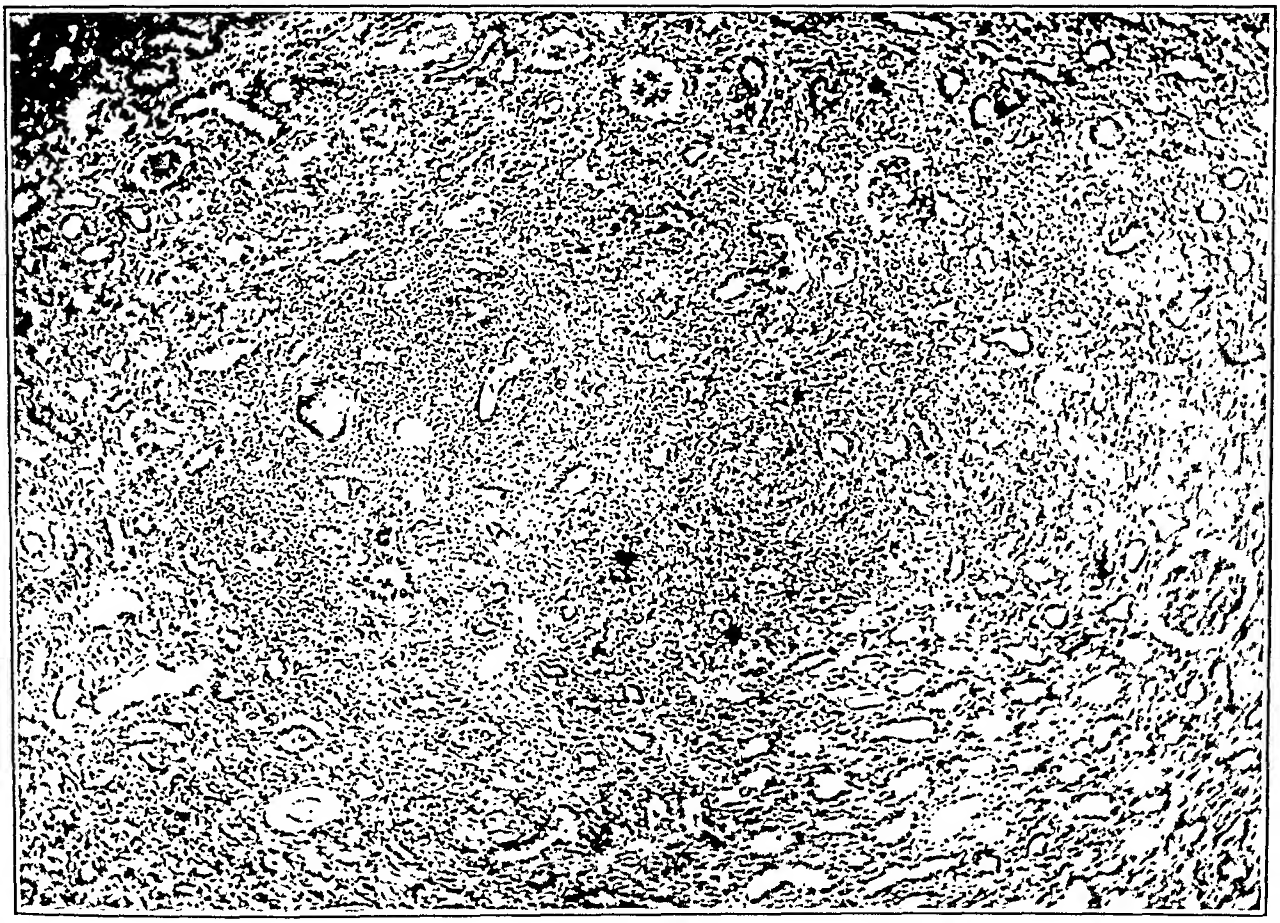

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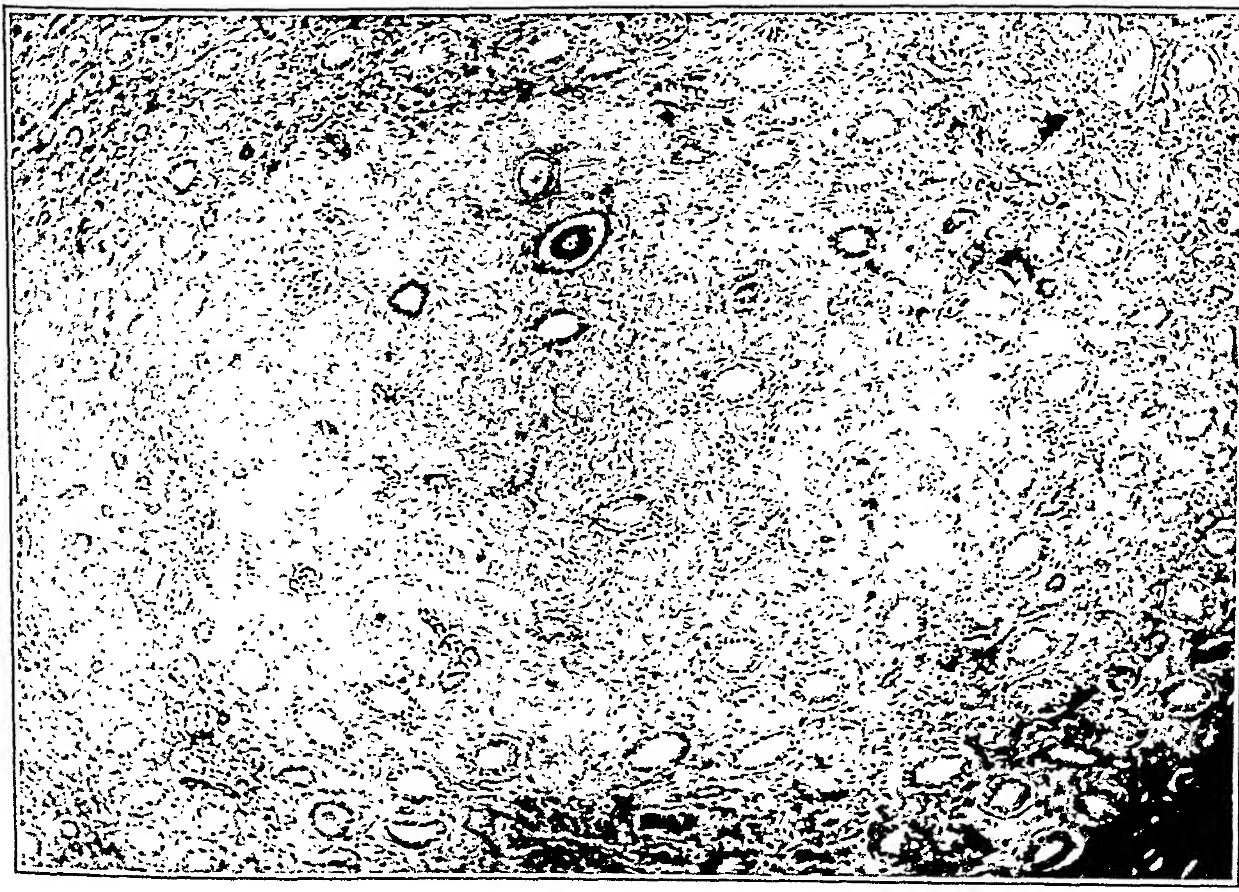

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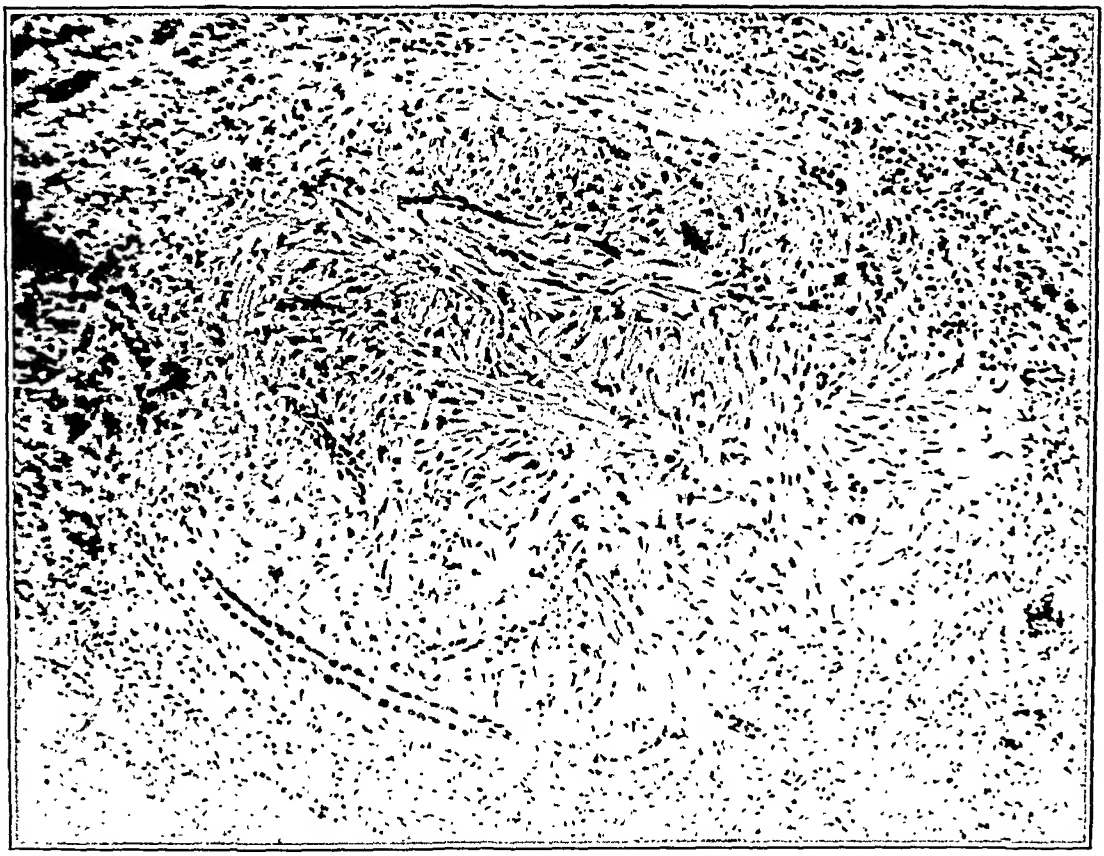
Medlar Renal Infection in Pulmonary Tuberculosis



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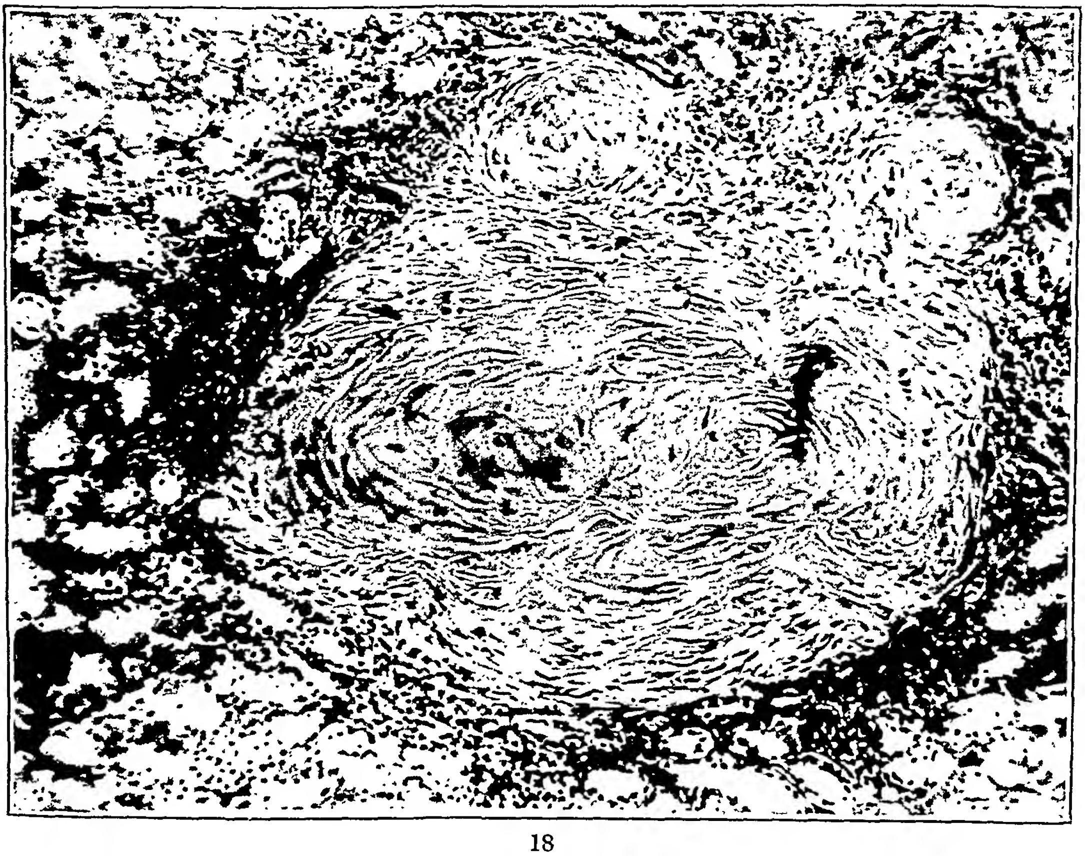

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EXPERIMENTAL PRODUCTION OF GENERAL PERITONITIS

WITH AN ANATOMIC STUDY *

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Three general methods are employed to produce experimental peritonitis: (1) intraperitoneal injection of microörganisms; (2) perforation of bowel; (3) ligation of the appendix. The results regarding life and the local peritoneal lesion vary with the species of animal employed.

White rats, guinea-pigs and rabbits succumb rapidly to intraperitoneal injection of fairly virulent bacteria. The peritoneum of the animals which survive for fourteen hours or longer following the intraperitoneal injection, may show a generalized hyperemia, slight deposition of fibrin and a small volume of a free gray fluid containing fibrin, polymorphonuclear leucocytes, some mononuclears and bacteria. In some instances the peritoneum shows no evidence of pathologic changes.

Benians,[1] using minute numbers of *Bacillus coli*, failed to produce death in the rabbit upon intraperitoneal injection; but when the microörganisms were mixed with gum tragacanth, death followed.

Cats and dogs seldom succumb to intraperitoneal injection even of very virulent microörganisms (Steinberg[2]). Ligation of the appendix (Costain,[3] Lehman and Copher[4]) produces death in forty-eight hours in a majority of the animals. Those dogs that survive have a localized abscess around the appendix (Steinberg; unpublished). Perforation of bowel occasionally results in death; if the animal survives, the peritoneum may show nothing or a few local abscesses.

When gum tragacanth is added to a culture of *B. coli* and injected intraperitoneally into dogs, the animals invariably succumb (Steinberg[2]). The peritoneum of these animals shows in six hours a severe hemorrhagic serofibrinous peritonitis. Gum tragacanth does not act

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merely as a foreign body (Steinberg and Goldblatt[5]) but probably possesses other properties by virtue of being a gum.

None of these methods results in a chronic or a healing acute general peritonitis such as is commonly seen in man.

Methods of Producing Experimental Peritonitis

1. Intraperitoneal Injection of Organisms
   (*a*) alone
   (*b*) with foreign bodies
   (*c*) with gum tragacanth
2. Perforation of Intestines
   (*a*) at various levels
3. Ligation of the Appendix
   (*a*) ligation only
   (*b*) ligation with perforation
   (*c*) ligation with injection of bacterial cultures into the lumen of the appendix
4. Intraperitoneal Injection of *B. coli* and its Antiserum

INTRAPERITONEAL INJECTION OF *B. COLI* AND ITS ANTISERUM

Steinberg and Ecker [6] produced an antiserum in rabbits against the soluble toxic substance of *B. coli*. Rabbits injected intravenously with this serum survive an otherwise fatal intraperitoneal injection of *B. coli*. The peritoneum of the rabbits receiving the antiserum intravenously and the *B. coli* intraperitoneally seldom shows any pathologic changes.

B. coli antiserum and a twenty-four hour broth culture of *B. coli* were mixed in equal volumes and incubated for one hour at 37 C. At the end of the hour, cultures of the mixture gave a profuse growth of *B. coli*.

Seven rabbits were used. Each animal received 30 cc. of the antiserum-culture mixture (15 cc. of antiserum and 15 cc. of culture) intraperitoneally.

Animals were killed at intervals of 14 hours, 18 hours, 1 day, 2 days, 3 days, 4 days and 11 days.

In fourteen hours a fibrinous exudate appeared on the diaphragm, surface of the liver, spleen and intestines. The large bowel was moderately hyperemic. Approximately 20 cc. of a grayish yellow, turbid fluid was found free in the peritoneal cavity. The fluid con-

tained fibrin, numerous polymorphonuclear leucocytes, some mononuclears and colon bacilli.

Microscopically, the peritoneal surfaces presented a layer of fibrin within which were enmeshed bacterial masses, mononuclear cells and leucocytes. Although the free peritoneal exudate contained many polymorphonuclears, the fixed tissue reaction was predominantly mononuclear in type.

In 18 hours, 1 day and 2 days, the fibrin increased in amount, binding by easily broken adhesions the omentum to the liver, loops of bowel to omentum and to each other (see Fig. 1).

In three days the diaphragmatic lymphatics were filled with bacterial masses, mononuclear cells and leucocytes which were also found between muscle bundles.

In four days the infection extended through the diaphragm and a fibrinous exudate appeared on its pleural surface (Fig. 2). The upper surface of the liver was adherent to the diaphragm by easily broken fibrinous adhesions. When pulled away, the surface of the liver was covered by a thick layer of fibrin. Microscopically, the fixed tissue exudate consisted predominantly of mononuclear cells, some of which (2 to 4 per field × 500) had become multinucleated (Fig. 3). The peritoneal and omental lymph nodes were hyperplastic. The omentum showed an infiltration of small mononuclear cells (Fig. 4). The free peritoneal fluid contained fibrin, many polymorphonuclear leucocytes and some mononuclears and but an occasional colon bacillus.

In eleven days the peritoneal cavity contained 100 cc. of a thick, gray fluid containing a great amount of fibrin, 52 per cent polymorphonuclears, 44 per cent small and large mononuclears and 4 per cent large mononuclear cells with vacuolated cytoplasm. No *B. coli* were seen and none was obtained on culture. Elevated grayish white patches several millimeters in diameter, some of them fused, were present on the diaphragm, omentum, liver, intestines and parietal peritoneum. Fibrous adhesions joined the omentum to the liver, stomach and loops of bowel (Fig. 5). Microscopically, the grayish white patches represented small abscesses surrounded by a thin layer of mononuclear cells, young fibroblasts and connective tissue (Figs. 6 and 7). At this period, fibrosis was established.

INTRAVENOUS IMMUNIZATION AND INTRAPERITONEAL INJECTION OF *B. COLI*

Benians[1] injected rabbits intravenously with small numbers of *B. coli*. Subsequent intraperitoneal injection of a fatal dose of *B. coli* produced no ill effects.

Three rabbits were each given intravenously 1 cc. salt solution containing one twentieth of a twenty-four hour agar slant growth of colon bacilli. This was repeated at the end of one week. Ten days after the last intravenous injection, 15 cc. of a twenty-four hour broth culture of *B. coli* (which proved fatal for a control rabbit in eight hours) was injected intraperitoneally into each of the rabbits. One rabbit was killed twenty-four hours later, the second animal forty-eight hours after the intraperitoneal injection and the third rabbit seventy-two hours after the injection.

None of these rabbits revealed any pathologic changes of the peritoneum.

INTRAPERITONEAL INJECTIONS OF (*a*) *B. COLI* ANTISERUM, (*b*) *B. COLI*, (*c*) *B. COLI* AND NORMAL SERUM

Three rabbits were injected with the antiserum against the soluble toxic substances of *B. coli*. Each rabbit received intraperitoneally 15 cc. of the serum. The animals were killed at intervals of 24, 48 and 72 hours. None of the three animals revealed any pathologic changes of the peritoneum.

Three rabbits were injected intraperitoneally with a twenty-four hour broth culture of *B. coli*. Each animal received 15 cc. of the culture. The three animals died in from eight to fourteen hours. The peritoneum of two animals showed a little free cloudy red exudate and slight hyperemia over the large bowel. The third rabbit, which survived for fourteen hours, had in addition a little fibrinous exudate on the diaphragm, liver and intestines.

Three rabbits were injected intraperitoneally with a mixture of normal rabbit serum and a twenty-four hour broth culture of *B. coli*, which was incubated for one hour at 37 C. Each animal received 30 cc. (15 cc. of normal serum and 15 cc. of *B. coli* culture). Two rabbits died eight hours later. One of these presented a slight hyperemia over the large bowel and a gaseous distention of the intestines; the other animal had a slight fibrinous exudate on the

peritoneal surface. The third rabbit survived and was killed on the fourth day. There was no free exudate in the peritoneal cavity. Cultures were negative for colon bacilli. The peritoneal surfaces were covered by small patches of fibrin. The infection extended through the diaphragm into the pleural cavities.

Summary

1. A method of producing acute and healing peritonitis is presented. This is accomplished by intraperitoneal injection of a twenty-four hour culture of colon bacilli together with an antiserum against colon bacilli.

2. Previous active immunization, and passive immunization induced by intravenous administration of antiserum, prevent the formation of peritonitis when *B. coli* cultures are injected intraperitoneally.

3. In the peritonitis produced under conditions of the experiment, the predominant type of cell differs in the free exudate within the peritoneal cavity from that in the fixed tissues. The polymorphonuclear leucocyte is the predominating cell in the free exudate and the mononuclear leucocyte predominates in the fixed tissues.

I desire to express my thanks to Professor H. T. Karsner for his many and helpful criticisms and his encouragement.

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DESCRIPTION OF PLATES

Plate 86

Fig. 1. Eighteen hour peritonitis. Fibrin on diaphragm; omentum adherent by fibrinous adhesions to ascending colon and stomach. Fibrinous exudate on parietal peritoneum.

Fig. 2. Pleural surface of diaphragm in four day peritonitis. Note the grayish white fibrinous exudate.

Plate 87

Fig. 3. Fixed tissue reaction in four day peritonitis. Note the predominant number of mononuclears and the two multinucleated cells, one in the center of the field, the other in the left lower corner. × 500.

Fig. 4. Omentum and lymph node in four day peritonitis. Note the enlarged germinal center, the hyperplasia of lymphocytes and the infiltration of omentum by small mononuclears. × 100.

Plate 88

Fig. 5. Eleven day peritonitis. Note the elevated patches on diaphragm, omentum and intestinal loop. Adhesions extend from omentum to liver, loops of bowel and stomach.

Fig. 6. Eleven day peritonitis. Note the small abscess and surrounding area. × 35.

Fig. 7. High power field of square in Fig. 6. Note the fibroblasts, connective tissue and mononuclear cells surrounding the abscess. × 500.

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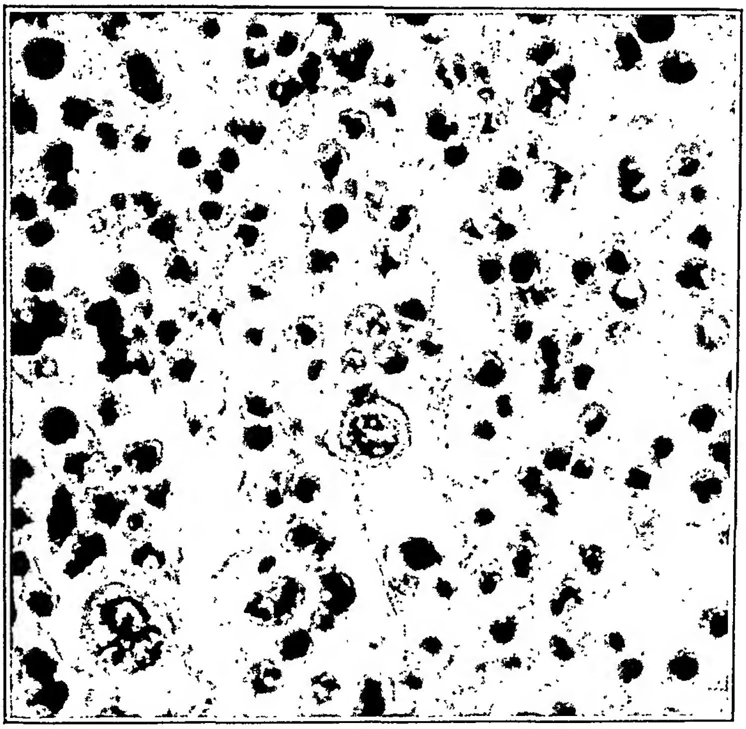

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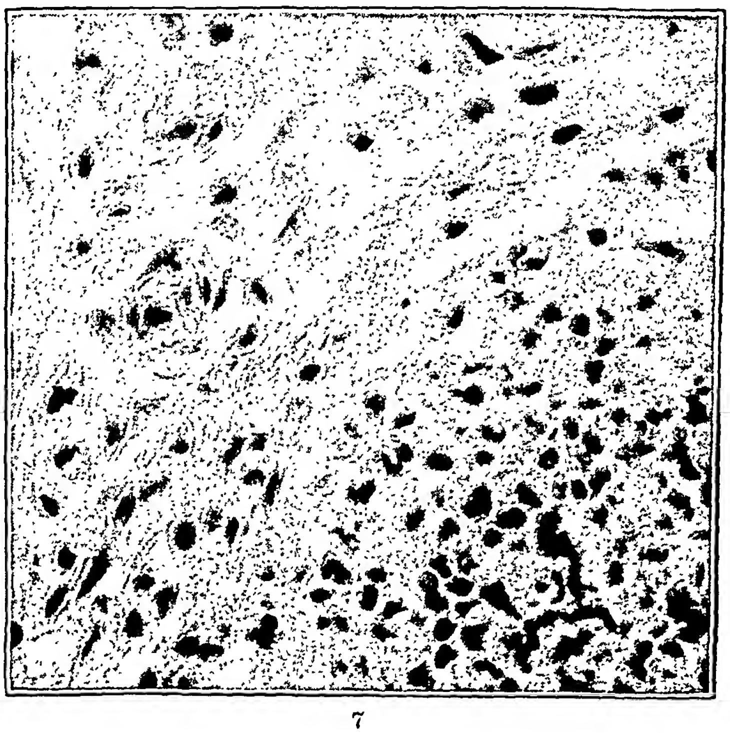

7

ON DIMINISHED RESISTANCE FOLLOWING SUPRARENALECTOMY IN THE RAT AND THE PROTECTION AFFORDED BY AUTOPLASTIC TRANSPLANTS *

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The resistance of suprarenalectomized animals to drugs and toxins has been studied by many investigators during the last thirty years. The earlier literature, reviewed and summarized in the recent papers of Lewis [1] and of Scott [2, 3], is unsatisfactory because of the lack of proper methods and control animals.

In rats between five and thirty days after suprarenalectomy, Lewis reported increased susceptibility to drugs, especially morphin to which these animals were from 400 to 500 times more sensitive than normal rats. Scott also reported marked diminution in the resistance of suprarenalectomized rats to morphin when tested from seven to fourteen days after operation. He found, too, that such animals succumb readily to bacterial intoxication, and showed that a dose of killed pyogenic cocci can be obtained that is invariably fatal to recently operated rats but never to controls. Jaffe and Marine [4] confirmed these results, injecting standard typhoid vaccine intraperitoneally, and showed that in a certain percentage of the animals compensation takes place in about nine weeks, when the rats withstand doses that would have proved fatal two weeks after operation. It will be shown that compensation, when it occurs, is dependent upon the hypertrophy of accessory suprarenal cortical tissue. Stewart and Rogoff,[5] Rogoff and De Necker,[6] and Rogoff and Ecker [7] state that, if tested after having fully recovered from the immediate operative effects, suprarenalectomized rats do not show increased susceptibility to toxins and drugs.

The purposes of this communication are: (1) to bring further proof that recently suprarenalectomized rats, fully recovered from the immediate operative effects, are highly susceptible to small doses of typhoid vaccine; (2) that as late as five months after operation, suprarenalectomized rats having no gross suprarenal accessory tis-

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sue are still very susceptible to vaccine; (3) that suprarenalectomized animals when compensated as regards resistance, invariably possess gross cortical accessory tissue; (4) that in the absence of gross accessories, autoplastic suprarenal transplants will protect suprarenalectomized rats against typhoid vaccine.

Methods

All rats were raised in the laboratory from tame albino stock. We previously described the care of our animals, the operative technic and the postoperative treatment.[8] Necropsies were performed on all rats and a careful search was made for suprarenal tissue. Standard typhoid vaccine containing one billion bacilli per cubic centimeter and heated to 38 C was injected intraperitoneally. This vaccine is a very satisfactory test substance because the minimum lethal dose for normal 250 gm. rats is between 20 and 30 cc., amounts much larger than those needed for testing resistance. Our results obtained from forty-six animals operated upon are as follows.

Resistance of Recently Suprarenalectomized Rats to Typhoid Vaccine

The fact that a large number of suprarenalectomized rats die spontaneously from suprarenal insufficiency during the first month must be considered in the interpretation of studies in susceptibility during this period. In a series of ninety-one rats, thirty-two died within thirty days after bilateral suprarenal removal, and approximately 85 per cent of these deaths occurred between the fourth and twelfth days, after which the critical period is past. If the injection of small doses of vaccine causes many fatalities after the twelfth day, these deaths may with reasonable certainty be attributed to the vaccine, and not to the moribund condition of the animals.

Eleven doubly suprarenalectomized rats, active and eating thirteen to sixteen days after operation, were injected with typhoid vaccine in doses ranging from 1 to 2 cc. Eight of these or 72 per cent were killed within nine hours by these doses which do not at all affect control rats. The suprarenalectomized animals became dull soon after the injection, some had tremors, coma ensued and death followed, preceded often by convulsions. These rats were killed by one-fifteenth to one-thirtieth of the minimum dose fatal to controls.

Table 1 concerns these eleven animals, ten of which gained weight during the postoperative period. No gross accessories were found at necropsy in the eight that died, and if present, they were microscopic and inadequate to afford protection. Of the three animals that survived, one was killed 129 days after suprarenalectomy by 5 cc. of vaccine; the other two survived 8 and 10 cc. 186 days after operation but when killed huge accessories were found.

Resistance to Typhoid Vaccine Long after Suprarenalectomy

A. *In rats without accessories.* It has been reported that the increased susceptibility of suprarenalectomized rats to toxins and drugs diminishes and completely disappears in time, but this is only partially true. When suprarenalectomized rats are followed for a year after operation, excluding those that die during the first month, the rest fall into two groups clinically and anatomically — those suffering from chronic suprarenal insufficiency with no gross accessories, and those biologically normal animals which invariably have large cortical accessory masses.

As late as five months after suprarenalectomy, the rats without accessories succumb to relatively small doses of vaccine, and Table 2 summarizes the data concerning twelve such animals which were injected between 42 and 129 days after suprarenalectomy and were killed by as little as 2 cc. of vaccine. These animals were lively and in good condition at the time of injection, though they showed signs of suprarenal insufficiency, particularly emaciation.

B. *In rats with accessories.* Table 3 presents data concerning eight suprarenalectomized rats that were injected between 65 and 186 days after suprarenalectomy with as much as 10 cc. of vaccine, and survived in every instance. In each animal, accessory tissue was found which on histologic examination showed typical, highly vascularized cortex.

The animals of this group quickly recovered from the immediate operative effects. After four weeks they were clinically indistinguishable from the non-operated litter and sex controls, at no time manifesting signs of suprarenal insufficiency. Unless distinction is made between groups A and B confusion on the subject of diminished resistance is inevitable.

TABLE I

Number	Sex	Time between suprarenalectomy and injection	Gain in weight after suprarenalectomy	Weight at injection	Amount of vaccine	Results	Remarks
		in days	*in grams*	*in grams*	*in cc.*		
35	F	15	30	115	1½	Died; 7 hours	Died from suprarenal insufficiency induced by typhoid vaccine
44	M	13	38	116	1½	Died; 9 hours	Died from suprarenal insufficiency induced by typhoid vaccine
45	F	13	39	106	1	Died; 9 hours	Died from suprarenal insufficiency induced by typhoid vaccine
31	F	16	30	123	1¾	Died; 9 hours	Died from suprarenal insufficiency induced by typhoid vaccine
32	F	16	23	113	1½	Died; 5½ hours	Died from suprarenal insufficiency induced by typhoid vaccine
33	F	16	19	105	1½	Died; 9 hours	Died from suprarenal insufficiency induced by typhoid vaccine
38	F	15	0	140	1½	Died; 9 hours	Died from suprarenal insufficiency induced by typhoid vaccine
43	M	14	37	101	1½	Died; 9 hours	Died from suprarenal insufficiency induced by typhoid vaccine
34	F	15	15	100	1½	Survived	Killed 129 days after suprarenalectomy by 5 cc. of vaccine; practically no abdominal fat; no accessories
36	F	15	13	177	2	Survived	186 days after suprarenalectomy survived 8 cc. of vaccine; killed 204 days after suprarenalectomy; 5 mm. and 1 mm. accessories
30	F	15	22	185	2	Survived	186 days after suprarenalectomy survived 8 cc. of vaccine; killed 210 days after suprarenalectomy; right 6 × 4 mm. accessory

TABLE 2

Number	Sex	Time between suprarenalectomy and injection	Weight at injection	Clinical condition at the time of injection	Amount of vaccine	Results	Remarks
		in days	*in grams*		*in cc.*		
34	F	129	198	Active; very slight emaciation	5	Died; 9 hours	Practically no abdominal fat; no accessories
53	M	110	223	Active; good condition	4	Died; 9 hours	Practically no abdominal fat; no accessories
59	F	121	177	Emaciated; good condition	$3\frac{1}{2}$	Died; 9 hours	Practically no abdominal fat; no accessories
56	M	108	200	Markedly emaciated; chronic insufficiency	2	Died; 8 hours	No abdominal fat; no accessories
68	F	114	164	Active	3	Died; 8 hours	No abdominal fat; no accessories
82	F	109	154	Active; emaciated	3	Died; 8 hours	No abdominal fat; no accessories
129	M	61	146	Active; somewhat emaciated	2	Died; 12 hours	Almost complete absence of abdominal fat; no accessories
147	F	47	123	Active; marked emaciation	2	Died; 9 hours	Practically no abdominal fat; no accessories
154	F	43	125	Active; considerably emaciated	2	Died; 7 hours	No abdominal fat; no accessories
156	M	43	150	Active; little emaciation	3	Died; 6 hours	No abdominal fat; no accessories
157	M	43	168	Active; no emaciation	3	Killed; dying	Complete absence of abdominal fat; no accessories
155	F	42	164	Active; slight emaciation	3	Died; 6 hours	Very little abdominal fat

TABLE 3

Number	Sex	Time between suprarenal-ectomy and injection	Weight at injec-tion	Clinical condition at the time of injection	Amount of vaccine	Results	Remarks
		in days	*in grams*		*in cc.*		
36	F	(1st) 133 (2nd) 186	221 221	Active; good condition Strong; no emaciation	5 8	Recovered Recovered	Killed 204 days after suprarenalectomy; left 5 mm. accessory; right 1 mm. accessory
39	F	(1st) 133 (2nd) 186	257 266	Excellent condition Excellent condition	5 10	Recovered Recovered	Killed 210 days after suprarenalectomy; right 6 × 4 mm. accessory
71	M	(1st) 107 (2nd) 167	247 292	Very good condition Active; slight emaciation	5 10	Recovered Recovered	Killed 198 days after suprarenalectomy; left 1 mm. accessory; right 3 × 2 mm. accessory
104	F	(1st) 66 (2nd) 120	174 187	Excellent condition Active; slight emaciation	4 8	Recovered Recovered	Killed 139 days after suprarenalectomy; considerable fat; right 2 × 2 mm. accessory
103	M	(1st) 66 (2nd) 120	215 291	Excellent condition Excellent condition	5 10	Recovered Recovered	Killed 139 days after suprarenalectomy; left 2 × 3 mm. accessory
106	F	(1st) 66 (2nd) 120	193 219	Excellent condition Excellent condition	4 10	Recovered Recovered	Killed 139 days after suprarenalectomy; right 5 × 4 mm. accessory
105	F	66	189	Excellent condition	4	Recovered	Killed 139 days after suprarenalectomy; left 3 × 3 mm. accessory
95	F	(1st) 68 (2nd) 120	163 180	Strong; active Not emaciated	4 8	Recovered Recovered	Killed 138 days after suprarenalectomy; right 6 × 4 mm. accessory

TABLE 4

Number	Sex	Time between autoplastic transplantation and injection	Weight at injection	Clinical condition at time of injection	Amount of vaccine	Results	Remarks
		in days	*in grams*		*in cc.*		
47	M	(1st) 114	285	Active	4	Recovered	Killed 204 days after suprarenalectomy; 4 good sized transplants; no accessories
		(2nd) 183	358	Excellent condition	10	Recovered	
52	F	(1st) 114	209	Active; good condition	5	Recovered	Killed 202 days after suprarenalectomy; 2 large transplants; no accessories
		(2nd) 183	234	Strong and active	10	Recovered	
50	M	(1st) 123	382	Excellent condition	7	Recovered	Killed 194 days after suprarenalectomy; 3 large transplants; no accessories
		(2nd) 174	443	Excellent condition	12	Recovered	
122	F	62	144	Eating; definitely emaciated	3	Died	Died after 22 hours; 3 tiny transplants; no accessories
124	F	(1st) 62	149	Active; slightly emaciated	3	Recovered	Died within a few hours; 3 large transplants; no accessories
		(2nd) 111	191	Good condition	8	Died	
127	F	(1st) 61	154	Active; slight emaciation	3	Recovered	Killed 128 days after suprarenalectomy; 3 large transplants; no accessories
		(2nd) 110	196	Good condition	8	Recovered	
128	F	61	151	Active; slightly emaciated	3	Recovered	Killed 128 days after suprarenalectomy; 3 large transplants; no accessories
143	F	47	161	Active; good condition	5	Died	Died within a few hours; 2 large and 1 small transplant; no accessories
144	F	47	158	Active; no clinical insufficiency	4	Died	Died within a few hours; 4 large transplants; no accessories
145	M	(1st) 47	190	Very good condition	6	Recovered	Killed 117 days after suprarenalectomy; 4 large transplants; no accessories
		(2nd) 97	280	Very good condition	10	Recovered	
148	F	44	176	Active; very slight emaciation	4	Died	Died within a few hours; 2 large transplants; no accessories
149	M	(1st) 44	188	Strong; good condition	4	Recovered	Killed 117 days after suprarenalectomy; 3 large transplants; no accessories.
		(2nd) 96	284	Excellent condition	8	Recovered	
150	M	(1st) 44	210	Excellent condition	$4\frac{1}{2}$	Recovered	Killed 117 days after suprarenalectomy; 3 large transplants; no accessories
		(2nd) 96	279	Excellent condition	10	Recovered	
151	M	(1st) 44	196	Strong; active	4	Recovered	Killed 116 days after suprarenalectomy; 2 large transplants; no accessories
		(2nd) 96	282	Active; slight emaciation	8	Recovered	
153	M	(1st) 44	181	Strong; active	4	Recovered	Killed 114 days after suprarenalectomy; 4 large transplants; no accessories
		(2nd) 96	243	Excellent condition	8	Recovered	

Resistance of Suprarenalectomized Rats with Autotransplants to Typhoid Vaccine

When the suprarenal is transplanted autoplastically, the regenerated cortex with neither its normal blood nor nerve supply, is nevertheless capable of protecting rats having no other gross suprarenal tissue in much the same way as hypertrophied accessories do. Fifteen transplanted animals are enumerated in Table 4, ten of which survived moderately large doses of vaccine. Four deaths were caused by the first injection, administered between the seventh and ninth weeks after operation. The transplants showed marked congestion and edema, and though regenerated, were as yet not sufficiently active physiologically to protect. The fifth rat was killed by the injection of 8 cc. of vaccine 191 days after transplantation, death being attributable to an overdose. The protection offered by transplants is clearly shown when Tables 2 and 4 are compared. All suprarenalectomized rats without accessories were killed by small doses of vaccine, while only 33 per cent of the transplanted rats without accessories succumbed, and these to larger doses. The transplant experiments offer further proof that resistance to vaccine is dependent upon the presence of active suprarenal cortical tissue.

Resistance of Normal Rats to Typhoid Vaccine

Young adult rats, weighing about 250 gm. withstood large amounts of vaccine. They suffered no appreciable effects from 3 or 4 cc.; after 5 cc. they had tremors but still reacted normally to stimulation; 10 cc. induced convulsions from which the animals recovered within a few hours. Twenty to 30 cc. sometimes killed and in these instances death was not due to the injection of large amounts of fluid for the peritoneal injection of 40 to 50 cc. of saline never kills.

Discussion

In acute infection and intoxication, pathologic changes regularly occur in the suprarenals varying in intensity from congestion and edema to hemorrhage and focal necrosis. Chronic infection and intoxication in animals are accompanied by a marked hypertrophy of the cortex with alteration in the distribution and amount of the lipoids. The significance of these pathologic findings is emphasized

by experimental studies in the resistance of suprarenalectomized animals.

We injected forty-six rats and found that the removal of both suprarenals invariably leads, in the absence of gross accessory tissue, to a considerable decrease in the resistance to typhoid vaccine. This is a consequence of the loss of some function of the cortex as shown by the facts that hypertrophied accessory rests protect suprarenalectomized rats against otherwise fatal doses of vaccine, and that autoplastic cortical transplants offer protection to animals having no other suprarenal tissue.

Rogoff and his co-workers report no significant change in the tolerance to morphin or tetanus toxin following suprarenalectomy, but they tested tolerance to substances which are extremely toxic even to normal rats. Their conclusions may be disputed, particularly in regard to morphin, if Table 1 of a recent paper [6] is analyzed. Of sixty-five animals injected between eight and thirty-six days after suprarenalectomy with doses of this drug ranging from 0.1 to 0.25 mgm. per gram body weight, which is less than the minimum lethal dose for normal rats, forty-four or about 67 per cent were killed.

Suprarenalectomized rats also show a lowered resistance to natural infections. As they become emaciated many of them develop snuffles, a condition from which normal rats or those with accessories or transplants are comparatively free, despite the circumstance that these animals are kept in the same cages under the same conditions.

Little is known of the mechanism of non-specific resistance to bacterial infection and intoxication, nor of the mechanism whereby suprarenal insufficiency affects the resistance of an animal. However when more knowledge of the function of the suprarenal cortex is acquired, it will be found that this tissue plays a vital part in the regulation of the bodily responses concerned with resistance to infections, infectious predispositions and general body well-being. It is our belief that the recovery from and the clinical course of severe infections, are dependent upon the proper functioning of the suprarenal cortex.

I wish to express my appreciation to Dr. David Marine at whose suggestion this work was undertaken.

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OBSERVATION OF FORMATION OF GIANT CELLS IN TURTLE BLOOD CULTURES *

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The histogenesis of the multinucleated giant cell found commonly in various inflammatory reactions and specific granulomas has long been a subject for discussion. Opinions differ both with regard to the type or types of the cells involved in giant cell formation and as to the nature of the process by which they are formed. Three theories have been promulgated to account for the multinucleated appearance: (*a*) mononuclear cells fuse to form multinucleated cells; (*b*) the nucleus of a mononuclear cell divides and the cell increases in size without the division of the cell itself; (*c*) giant cells are not true cells but are agglomerations of cells which adhere to each other and retain their individuality, or are merely agglomerations of cells included in necrotic masses.

Each one of these theories has had supporters. The earlier work was done on fixed tissues. Mallory [1] in 1911 stated that foreign body giant cells formed by fusion, contrasting them with true tumor giant cells which he said formed by multiple mitosis. Forbes [2] in 1909 in experimental work on the development of foreign body giant cells in rabbits injected with agar remarked that the endothelial cells coalesced to form giant cells. Duval and White,[3] discussing the histology of glanders, took the opposite view. They concluded that the giant cells in their lesions formed by division of the nucleus of the endothelial cell and not by fusion.

Since it has been possible to study living cells by tissue culture methods, Lambert [4] in 1912 reported the formation of giant cells *in vitro* using chick embryo spleen. He noted the fusion of cells resulting in the formation of a thick giant cell. In 1921 Lewis and Webster [5] reported the formation of giant cells in cultures from human lymph nodes. They were led to believe that the giant cells arose from large wandering cells by amitosis. They observed one case of amitosis but no signs of mitosis. Further, M. R. Lewis and

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W. H. Lewis[6] in work done with hanging drop cultures asserted definitely that giant cells (of the Langhans type) did not arise by fusion. They were of the opinion that the cells arose by division of the nuclei without division of the cytoplasm. Since then, however, Lewis and Brüda[7] cultivated tissue from a white blood cell tumor of the rat and they stated definitely that they observed fusion of epithelioid types of cells to form giant cells.

Medlar[8] recently advanced the opinion that the giant cells were either agglomerations of adherent cells or collections of cells included in necrotic masses. A similar interpretation was suggested as early as 1885 by Baumgarten and Weigert who believed the giant cells were necrobiotic structures from the beginning (Quoted by Hektoen[9]). Hektoen[9] reviewed the literature in 1898 and while he remarked that he was not prepared to give an opinion as to the mode of formation of the giant cells, he did note that the cells, under certain conditions, separated into small living cells, and for that reason, believed they were living structures.

TECHNICAL METHODS

Turtle blood was used because the experiments could be carried on at room temperature. The most commonly used preparation was the simple hanging drop of blood prepared similarly to the method described by Lewis.[10] Blood was removed from the heart by means of a syringe and a fair sized drop was placed in the center of a clean cover glass. The cover glass was then inverted and sealed over a hollow ground slide with a vaseline-paraffin mixture. A similar preparation was made after the turtle had been injected with India ink. Five drops of India ink (Higgins) were added to 10 cc. of normal salt solution, the suspension was filtered twice and 0.5 cc. was injected directly into the circulation through the heart. Preparations were made one hour after injection. A third type of preparation was made using a modified Locke-Lewis solution. A drop of this solution was placed upon the cover glass and then a drop of blood was added. In one series of experiments the Locke-Lewis solution was added on the fifth day of incubation to plain blood preparations. A fourth type of preparation was made by Sabin's[11] method using both neutral red and Janus green. These preparations, while not hanging drop preparations, were used primarily for detailed morphologic study.

EARLY CULTURES

The drop after several hours shows a clear peripheral zone with the blood cells collected in the center. At the periphery of the cell area the white cells have gathered and the entire mass is clotted. Usually, within two days, wandering cells are seen advancing into the clear peripheral zone. These cells are large, irregular in shape, actively motile and have longer and shorter protoplasmic projections. The latter vary continuously producing changes in the shape and position of the cell. The cytoplasm is finely granular and contains rod-shaped mitochondrial granules. The granules occupy almost all of the cytoplasm in the cells of earlier preparations. The nucleus is a bland ovoid body which occupies no constant position in the cell; in fact, it changes its position with the movements of the cell. In the India ink preparation, black granules are present in most of these cells usually in the neighbourhood of the nucleus. These cells seem generally to migrate toward the periphery of the clear zone. In many of the cultures on the fourth day, large irregular multinucleated giant cells are seen at the very edge of the drop, clinging quite closely to the cover slide. They are continuously altering their shape. These cells contain from four to twelve large clear nuclei arranged irregularly throughout the cytoplasm. Nucleoli are present in most of the nuclei. Granules of varying sizes, mitochondria and vacuoles are seen in the cytoplasm. Often when red cells are near (and they are always undergoing disintegration when in the neighborhood of the giant cells), they are phagocytized and found lying within the cytoplasm of the giant cell. When India ink is used in the preparations, it is found distributed irregularly in small masses in the cytoplasm.

FUSION

The fusion is observed at the height of activity which occurs most commonly on the fourth day, although in some preparations great activity persists until the eighth or ninth day, and in one culture until the twenty-fifth day. Multinucleated giant cells are seen at the edge of the drop undergoing active changes. These cells send out projecting cytoplasm in their activity and seem to attract the surrounding cells. Large irregular wandering cells in the neighborhood also produce protoplasmic processes, as they advance toward the giant cell. When a protoplasmic projection of a wandering cell

comes in contact with the protoplasm of the giant cell which is usually also projected at that point, the protoplasmic processes widen, become one, and the contents of the wandering cell flow into the giant cell, leaving no evidence whatever of the wandering cell. The giant cell, observed for hours at a time after such a phenomenon, carries on its activity as before uninfluenced save for its increase in size. Often two mononucleated wandering cells fuse in the same fashion as they advance toward the giant cell. The new binucleated cell then assumes activity as an independent giant cell or subsequently fuses with another giant cell.

There are instances in which quiescent spherical mononuclear cells, from one to two times the size of red blood corpuscles, are seen to become motile and to fuse with wandering cells or with giant cells. The steps in this transformation are definite and fairly uniform. When first observed the cytoplasm of the spherical cells is undifferentiated and the nucleus is obscured. Suddenly the cell develops an exaggerated Brownian movement, sends out pseudopodia, develops definite rod-shaped mitochondria, discloses its nucleus and begins active ameboid motion, spreading itself so that it appears identical with other wandering cells. This cell then appears to be attracted to another wandering cell and fuses with it, or advances toward a giant cell and fuses with it. Often after several moments of active movement without fusion this cell resumes its spherical undifferentiated form and becomes quiescent. Some of the cells undergo transformation two or three times and then finally fuse with another wandering or giant cell.

FRAGMENTATION

The giant cells on observation over days do not increase in size beyond a certain point. They continue to fuse with other cells, but at intervals parts break from the main cell and move away as irregular wandering cells. On rare occasions they assume a spherical quiescent appearance. Occasionally the wandering cells return and fuse with the main cell body. At times the giant cells in their activity separate into two or three multinucleated parts. In instances they fuse again, at other times they wander away and remain as individual independent giant cells.

After several days of such activity the cells become still, develop vacuoles of varying sizes, finally break up and disappear. The

time when this disintegration occurs is never constant. Some cells break up on the twelfth day while one preparation exhibited excellent cell activity on the twenty-sixth day.

Discussion

There can be no doubt as to the formation of multinucleated giant cells by fusion in our experiments. We do not wish to indicate that such cells may not also be formed by amitosis of the nucleus with division of the cells, but in hours of constant observation we failed to detect such a process. The activity within the dense portion of the drop was never observed in the living state; however, stained preparations gave no evidence that nuclear division occurred.

In our work we have confined ourselves to observations on the development of multinucleated cells from mononuclear white cells in cultures of turtle blood *in vitro*, with the single purpose of determining whether or not such mononuclear cells may fuse to form multinucleated cells. We made no attempt to determine the origin of the mononuclear white cells under observation.

The fact that whole multinucleated cells were seen to migrate as entities and that no protoplasmic divisions were perceptible either during cultural stages or after fixation and staining, practically precludes the possibility of the multinucleated masses being agglomerations of separate cells.

Difficulties in comparing mammalian and reptilian blood were encountered both with films fixed and stained, and films prepared according to Sabin's [11] technic. The type cell entering the reaction was not identified as to its origin. We were content in recognizing it as a mononuclear wandering cell with phagocytic properties, evidenced by the ingestion of India ink particles.

The India ink preparations were not different from the simple preparations save for the presence of the black particles in the wandering cells and in the giant cells.

The results of the experiments with Locke-Lewis solution were not as satisfactory. A few small multinucleated giant cells were formed in some of the preparations, but the activity was retarded.

Conclusions

1. Only large mononuclear wandering cells were seen to enter into the formation of giant cells.

2. Multinucleated giant cells were observed to form by fusion in hanging drop cultures of turtle blood.

3. Quiescent non-granular mononuclear types of cells were observed to take on ameboid activity, to show mitochondrial granules and to participate in fusion phenomena.

4. The multinucleated giant cells behaved like true cells, showed no partitions and were not merely coherent masses of single cells.

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DESCRIPTION OF PLATES

Plate 89

Fig. 1. Giant cell with two nuclei showing long protoplasmic processes; elongated wandering cell in neighborhood advancing toward large cell.

Fig. 2. Beginning fusion of two large wandering cells.

Fig. 3. Giant cell from four-day culture.

Plate 90

Fig. 4. Camera lucida sketch of giant cell in eight-day culture; note wandering cell approaching giant cell.

Fig. 5. Same cell nineteen hours later; spherical forms shown, one just having taken on ameboid activity. Giant cells beginning to break.

Fig. 6. Same cell one hour later; the parts breaking further.

Fig. 7. Giant cell activity on twenty-fifth day.

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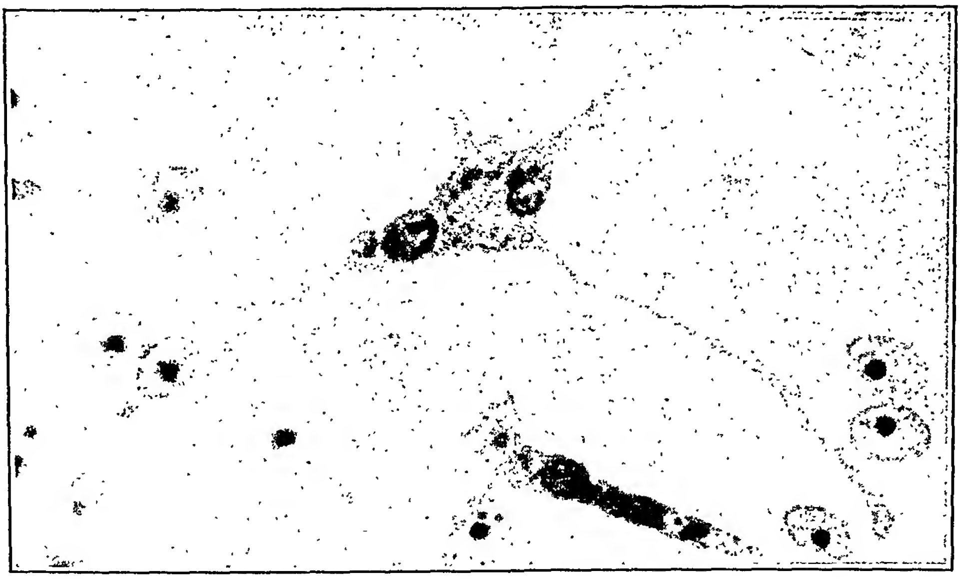

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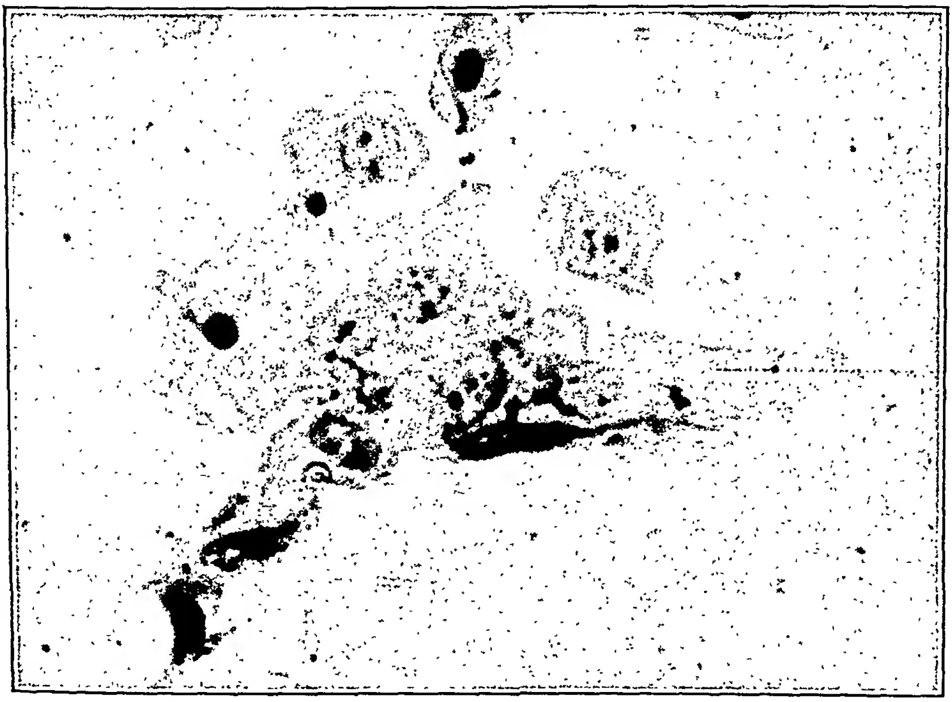

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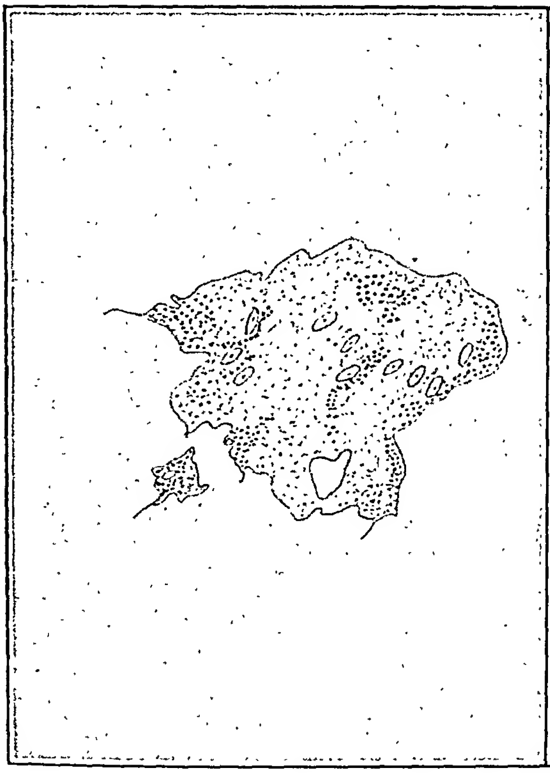

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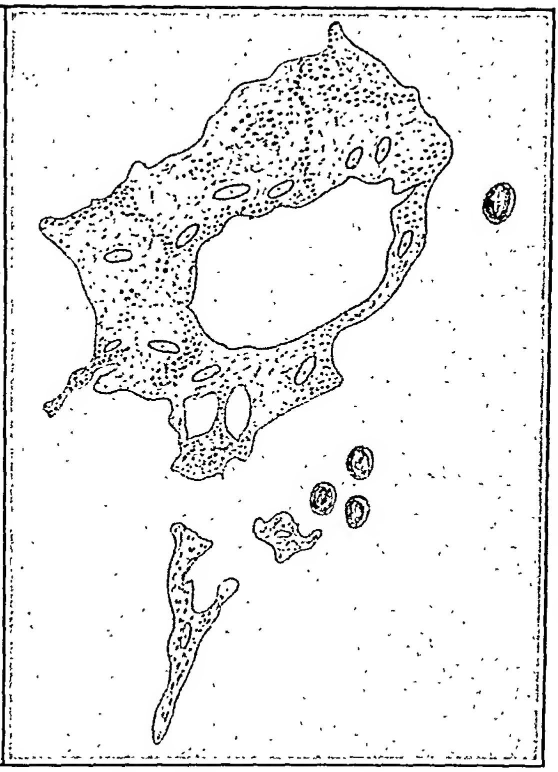

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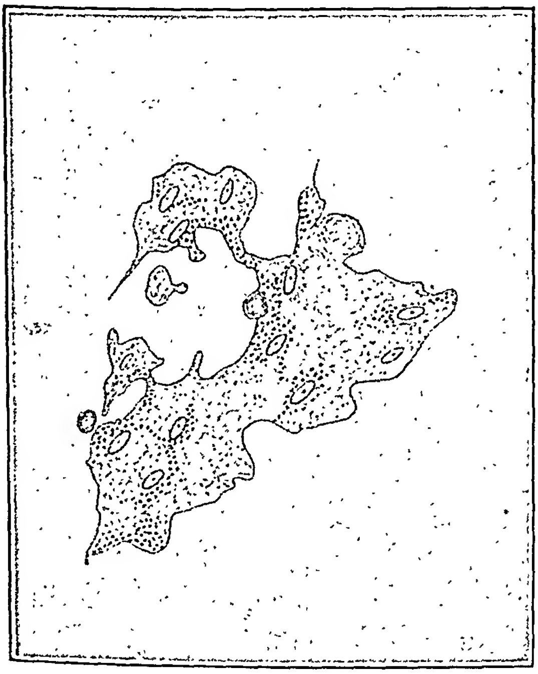

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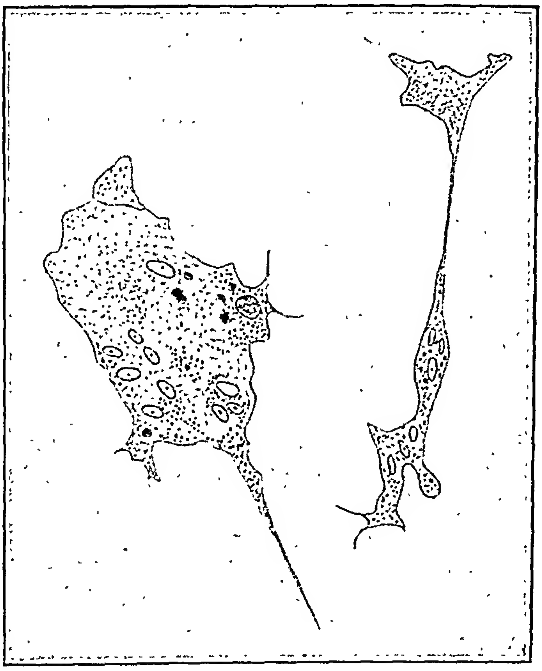

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SCIENTIFIC PROCEEDINGS OF THE TWENTY-SIXTH ANNUAL MEETING

OF THE

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

ALBANY, NEW YORK

APRIL 2 AND 3, 1926

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

SCIENTIFIC PROCEEDINGS

A BACTERIOLOGIC STUDY IN CASES OF POSTOPERATIVE THROMBOSIS AND EMBOLISM. E. C. ROSENOW, Rochester, Minn.

Abstract. Partial tension cultures have been made in five cases of postoperative pulmonary embolism secondary to thrombosis of iliac or femoral veins and one case of portal thrombosis. The thrombus and embolus were removed in as sterile a manner as possible, washed repeatedly in large amounts of sodium chloride solution, or the surface was seared with a searing blade. Emulsions in broth or sodium chloride solution made by grinding small pieces of the thrombus in a sterile mortar with sand, were inoculated into tall tubes of melted glucose brain agar, glucose brain broth and other mediums. Similar cultures were made from the blood and from the pipettings from the spleen, liver and kidneys. Necropsy in all of these cases was performed within twelve hours after death. A diplococcus similar to the one obtained twelve years ago in similar cases in Chicago was isolated from the embolus and thrombus in each of the five cases of pulmonary emoblism, and from the thrombus in the case of portal thrombosis. A staphylococcus was also obtained from emulsions in two cases. The number of colonies of the diplococci was not large, never more than 500 per gram of thrombus. The colonies could be seen growing on the edges of the pieces planted into the soft glucose brain agar. Primary aerobic cultures on blood agar were always sterile but subcultures grew readily on aerobic blood agar plates. The blood from the heart, liver, spleen and kidneys was sterile in four cases. The diplococcus was obtained from the blood in one instance only. In one case of sudden death from pulmonary embolism following amputation of the breast for ulcerating carcinoma and in which marked cellulitis with septic fever occurred, a hemolytic streptococcus was isolated from the wound and blood in pure culture and from the pulmonary embolus and iliac thrombus in mixture with the diplococcus. In the case of portal thrombosis associated with peritonitis following gastrectomy for carcinoma of stomach, *B. coli* and *B. welchii* were found in addition to the diplococcus. Cultures from the blood yielded *B. coli.* The organism isolated from the thrombus was much alike in the different cases. It is a gram-positive diplococcus; its size, shape and grouping resembles the pneumococcus closely but it is free from a capsule and the individual cocci are more nearly round. It produces small dry nonadherent colonies on horse blood agar plates, which are usually surrounded by a green or brownish green zone of partial hemolysis. The diplococcus is of low general virulence, it does not grow well in citrated blood *in vitro*, it shortens the coagulation time of the blood markedly, and it tends to incite thrombosis in animals following intravenous and other injections. It has been demonstrated microscopically in the thrombi produced

experimentally and in the thrombus of pulmonary embolus of all but two of twenty-five patients that died of postoperative pulmonary embolism.

Two of the strains fermented inulin and salicin; one of these also fermented mannite; the rest did not ferment these sugars. All strains fermented glucose, lactose and raffinose.

(No discussion.)

FURTHER OBSERVATIONS ON THE STREPTOCOCCUS TOXINS AND THEIR ANTITOXINS AND THE STANDARDIZATION OF THERAPEUTIC SERUMS. Mary B. Kirkbride and (by invitation) Mary W. Wheeler, Albany.

Abstract. Toxicity tests on goats indicated no fundamental qualitative or quantitative differences in the toxins of hemolytic streptococci from scarlet fever or from other infections; toxicity tests on human subjects were apparently not as dependable, as the same toxins induced variable reactions in different individuals.

As a result of titration tests on goats with eighty serums including trial bleedings, confirmed with tests of sixteen serums on human subjects, a method for standardizing scarlet fever antistreptococcus serums on goats has been developed. This depends upon comparative tests of the test serums with a standard serum against a dose of a standard toxin, made at the same time, on the same animal under carefully controlled conditions.

(Discussion by Dr. W. H. Park, New York.) I am just wondering whether the observations of Miss Kirkbride and Miss Wheeler are correct in that goats would show differences between the toxins of different strains when human beings might not be so well used. It would seem to me that the goat could not be used for that purpose as well as human beings. There is no question that in the persons who showed marked difference in the reactions to the Dochez and the Dick toxin that there was a difference between the component toxins of those two strains, while on goats there would apparently be no difference. I think it would be like testing for similarity of agglutinins and not using the absorption method. With the ordinary agglutination method many different cultures would react alike where with the absorption method they would differ. The human being therefore would be a more accurate and real test for differences than the goat.

(Miss M. W. Wheeler, Albany, closing.) In answer to Dr. Park's question — it might be possible to distinguish certain qualitative differences in the toxins of different scarlet fever streptococci more readily by the human test than by the goat test. The human test, however, apparently cannot be relied upon for differentiating the specific scarlet fever streptococci from other hemolytic streptococci, since we have found that individuals frequently vary in their reactions to toxins of different strains of streptococci isolated from typical cases of scarlet fever.

THE PREPARATION AND CONCENTRATION OF SCARLET FEVER ANTITOXIN AND ITS CLINICAL APPLICATIONS. John F. Anderson and (by invitation) George F. Leonard, New Brunswick, N. J.

Abstract. This paper reports the results of the immunization of eighty-four horses for the preparation of streptococcus scarlet fever antitoxin prepared according to the methods described and developed by the Dicks. The horses were immunized chiefly by the injection of either filtered broth cultures of scarlet fever streptococci or by the injection of fresh cultures of scarlet fever strepto-

cocci which had been passed through a Sharples centrifuge. The horses received toxin testing as high as 100,000 skin test doses per cc. Five different strains of scarlet fever streptococci were used in connection with the immunization of the horses. The antitoxin was concentrated by the Banzhaf process.

A report was made of 130 severe and moderately severe scarlet fever cases in three institutions in three different states which were treated with this antitoxin, as compared with the results in eighty-four cases in the same institution who received no antitoxin. Among the 130 cases, some of which were severe with marked toxemia, there were no deaths, and only 4.6 per cent developed complications, none of a severe character or requiring surgical intervention. Of the eighty-four mild cases, without antitoxin, there were no deaths, but 28.6 per cent had complications and sequelae of varying degrees of severity. Some of the complications were of a grave character, requiring surgical intervention, and consisted of nephritis of a serious grade, otitis media, mastoiditis and suppurating cervical adenitis.

The amount of antitoxin as given in the three institutions is shown in the following table:

Dosage

Hospital	Maximum	Minimum	Average
A	520,000 neutralizing doses	80,000	221,750
B	300,000 " "	75,000	137,850
C	600,000 " "	200,000	256,800

The results as reported are summarized as follows:

Specific scarlet fever antitoxin may be prepared by the immunization of horses with filtered toxin.

Such antitoxin is specific against scarlet fever occurring in widely separated sections of the United States.

A properly prepared and standardized antitoxin is effective as a prophylactic when used in adequate doses.

When used for passive immunization, it should be given in not less than one half of the average therapeutic dose.

A properly prepared and standardized scarlet fever antitoxin is effective in the treatment of scarlet fever, saving life and reducing the severity and frequency of complications.

(Discussion by Dr. W. H. Park, New York.) It happens that in our work we have used only a very few horses but one-half of these horses have been on the Dochez method and one half on the so-called Dick method. The most potent horse was on the Dick method but one almost as good was on the Dochez method and the fact that we have been using the serum made by both methods with excellent results, seems to me to show that either method will give highly potent and useful serum. Apparently one depends rather more on a suitable horse than on the method. As far as the annoyance goes, there is very little annoyance if you have the scarlet fever horses in separate stables. There is very little trouble from the abscesses. They are small and in a week or so heal up entirely.

There is another point I want to discuss and that is the question as to whether we should inject the serum to prevent scarlet fever in those who have been exposed. I agree with Dr. Anderson that practically every case will be protected for ten days or two weeks. On the other hand, we have the difficulty that contagion will probably persist longer than that in any institution or a family and

you will have to repeat the dose. Unquestionably you get a greater serum reaction from the present refined serum than from the refined diphtheria antitoxin, and there is still a greater difference if unrefined serum is used. This is probably due to the fact that there has been no time to age the serum. Scarlet fever is not a very contagious disease. In New York City about one in ten of children exposed in families will develop scarlet fever. I prefer, therefore, to do an immediate Dick test and then if the child or person develops a sore throat or the slightest clinical evidence of scarlet fever, then give a therapeutic dose of the serum. I have had experience in certain institutions and families where we have used this method and they have had very little reaction. So far, in over 100 exposed cases so treated there has only been one case of scarlet fever develop.

(Discussion by Miss M. B. Kirkbride, Albany.) In connection with the standardization and the production of the antitoxin, although we have had very few horses, we have had a few on the Dick method and a few on the Dochez method. Our highest titred serums, however, have been obtained with a combination of the two methods. One cubic centimeter of serum neutralizes, I think, from 20,000 to 30,000 skin test doses of toxin.

(Discussion by Dr. E. C. Rosenow, Rochester, Minn.) The results following administration of antitoxin in two cases of scarlet fever which I have seen will be of interest. Both occurred in children of one family. The fever and rash disappeared promptly following administration of the antitoxin on the second and third day, respectively. Six days later both patients developed nausea and vomiting, diarrhoea, severe gastro-enteritis, high fever, marked tenderness of abdomen, and one a pelvic abscess due to scarlatinal hemolytic streptococci. The development of the abscess was interpreted by Dr. Helmholz as secondary to enlarged retroperitoneal lymph glands because the antitoxin was given deep into the gluteal muscles. Whether this interpretation is correct or not is, of course, problematic, but in the light of these findings I would warn against the giving of scarlatinal antitoxin intramuscularly where the deep lymphatics of the peritoneal cavity may become involved.

(Discussion by Dr. James Ewing, New York.) I recently saw a child with progressive, general enlargement of the lymph nodes, severe anemia and a blood picture of aleukemic leukemia. The parents were convinced that the disease had resulted from a preventive dose of scarlatinal antitoxin. The history was consistent with this interpretation. I have heard of one other case of this same type.

While I am very skeptical about the relation between the antitoxin and the progressive anemia in these cases, both of which were fatal, I think such observations should be recorded, since, if they accumulate in sufficient numbers, they may possibly be of significance.

(Dr. J. F. Anderson, New Brunswick, N. J., closing.) In answer to Dr. Park's comments I would state that I have never advocated the routine use of antitoxin in diphtheria or scarlet fever as a preventive measure. I think it is an unsound and unscientific way of dealing with the situation. Nevertheless, there are many doctors who still use diphtheria antitoxin as a routine measure in the case of exposure of individuals to diphtheria and there are certainly a very large number who are doing the same thing with the scarlet fever antitoxin. As a matter of fact I think there is twice as much of the preventive dose of the scarlet fever antitoxin distributed as there is therapeutic. I agree with Dr. Park that what should be done in such cases is very close observation of the child and at the first evidence of infection a full sized dose of antitoxin be given.

If, however, the physician or family insist upon the administration of antitoxin as a prophylactic measure the proper procedure is certainly to begin immunizing with the toxin two weeks later in order to bring about active immunity.

In regard to Miss Kirkbride's comments — we have never used the Dochez method but we have been using the method suggested by Zinsser and we believe that it has many points of advantage. That is the method in which you use oxalated blood inoculated with the streptococcus and grown overnight. Immediately before injection add a proper amount of calcium chloride to bring about clotting after injection into the animal. We have five different groups of horses, not less than five in any group which are under different methods of immunization. We have a group given filtered toxin, another group is given filtered toxin plus injections of live cultures. Another group is given the Zinsser method alone. Another group Zinsser method plus the subcutaneous injections of the filtered toxin. In the course of the next few months we will have something that will at least indicate to us whether any of these various methods offer much over another.

STANDARDIZATION OF SCARLET FEVER ANTITOXIN. William H. Park, New York.

Abstract. There are difficulties met with in carrying out the Dick method of standardizing scarlet fever antitoxin. The subjects must be human beings who while strongly positive to the Dick test are not sensitive to the horse serum containing the antitoxin to be tested.

Some persons who show the same degree of reaction to the Dick test as others do not respond equally. Unless a great many tests are carried out it is impossible to test the potency of a serum within fine limits.

Certain breeds of goats have been used by Kirkbride and Wheeler with success. They respond to even smaller amounts of toxin than human beings. Some of them are sensitive to horse serum and all become so a few days after testing. The great advantage is that we are relieved from testing on human beings. The potency of a serum can be tested within broad limits. The Schultz Charlton blanching phenomenon can be used for testing the potency of a serum as brought out by Blake and Dochez. On the chart you see the results of using four serums in different dilutions. One cc. of a 1:100,000 dilution of a serum that neutralized 50,000 Dick skin test doses of toxin caused definite blanching in suitable cases. This method has its difficulties. It is necessary to have access to scarlet fever cases with suitable rashes, which will not receive serum treatment. An evenly distributed erythema on the first or second day of its appearance is the best and it must remain bright for at least eighteen hours. Even then different rashes give different potency tests. Older rashes are not so good and give lower readings. Unless a great many tests are made only a fair estimate of potency can be made.

All these methods, however, give tests of sufficient accuracy to be used by us in the treatment of cases. A number of us are now arranging to have a large amount of serum standardized so that the standard serum can be compared with the serum to be tested. This will make for more accurate results.

(No discussion.)

FACTORS INFLUENCING THE POTENCY OF SCARLATINA TOXIN. Anna W. Williams, New York.

Abstract. The toxins reported here were all prepared one year or more ago. We have observed the effect of the different factors mentioned both on the potency immediately after preparation and on the permanency of that potency.

1. Amount of peptone. Two per cent peptone (Digestive Ferments proteose or Park Davis) gives a stronger toxin than 1 per cent but the strength of the toxin drops more rapidly and approaches in time the same potency as the 1 per cent.

2. Blood. The same thing is true for blood. All bloods tried give a stronger toxin than the same lot of broth without blood, but the drop is proportionately great.

3. Reaction. With a less alkaline reaction (pH 9.4) the toxin is stronger than with a more alkaline one (pH 8.2) up to the fifth day; after that up to the tenth day the more alkaline medium gives a stronger toxin.

4. Time of growth in incubator. The longer growth (7 to 10 days) has given a more stable toxin.

5. Animal passage. Strains greatly increased in virulence by mouse passage make a much stronger toxin than the original strains but the drop in toxicity is greater. However, their final potency remains appreciably greater than that of the toxin from the original strains.

6. The drop of potency of these very strong toxins is variably rapid. In about two months they may drop 50 per cent — in another month a half more, and after that the drop is slower.

(No discussion.)

THE EFFECT OF CINCHONICS ON PNEUMOCOCCI. I. THE INFLUENCE OF QUININ UPON PROLIFERATION AND AUTOLYSIS OF PNEUMOCOCCI, WITH SPECIAL REFERENCE TO THE EFFECT OF HYDROGEN ION CONCENTRATION. Meyer Solis-Cohen, Philadelphia.

Abstract. The germicidal action of the quinin salts against pneumococci increases with increase in alkalinity. An increase in effectiveness begins to become evident at the reaction of normal blood (pH 7.4) but its maximum effect is shown in a reaction more alkaline than this.

An interesting phenomenon becomes evident when the effect of quinin in buffered broths of varying hydrogen ion concentration is studied. In broths of pH 6.3 to 6.8 of such acidity that growth of pneumococci normally does not occur, pneumococci are stimulated to growth within a certain zone of quinin dilutions (1:15,000–1:50,000 quinin hydrochloride, depending on the pH). No growth occurs in concentrations of quinin higher or lower than those in this zone.

In a study of the effect of quantity of organisms upon the pneumococcidal action of quinin, it was noted that upon diluting the seed culture a dilution was eventually attained that failed to grow in the control tube of broth but that was stimulated to growth in weak dilutions of quinin.

Especially with optochin, growth of pneumococci in very high dilutions was less favorable than in the control tube; in moderate dilutions, growth was as marked or more marked than in the control tubes; in low dilutions inhibition and death of the organisms became evident.

In the presence of quinin, autolysis of pneumococci within a period of twenty-four hours is extended to a range of hydrogen ion concentration more alkaline (between pH 5.5 and 7.6) than that in which dissolution occurs in the absence of quinin (between pH 5.5 and 6.8). This phenomenon is pronounced in a concentration of 1:4000, is still markedly evident in a concentration of 1:20,000 but becomes less evident in a concentration of 1:100,000 and practically non-existent in a dilution as great as 1:400,000.

There is, furthermore, a tendency, particularly in the alkaline broths, for autolysis to occur at an earlier period in dilutions of quinin than in the broth control tubes.

(Discussion by Dr. C. W. Jungeblut, Albany.) I have seen in one of these charts that pneumococci which had been cultivated in the very high dilutions of optochin apparently did not grow as well as those from the more concentrated dilutions. This fact which, *a priori*, is difficult to understand, might be explained by the phenomenon which Schnabel described five years ago. He found that pneumococci after having been exposed to very high dilutions of optochin, such as one to ten or one to twenty million, would exhibit at the next passage a specifically increased sensitiveness to the drug, while those obtained from the lower concentrations became fast.

(Dr. Meyer Solis-Cohen. No reply.)

THE EFFECT OF CINCHONICS ON PNEUMOCOCCI. II. CONTRASTED BEHAVIOR OF PNEUMOCOCCI TOWARD QUININ AND OPTOCHIN IN RELATION TO DRUG FASTNESS. Meyer Solis-Cohen, Philadelphia.

Abstract. Resistance of pneumococci to optochin may be developed very quickly. Within an experimental period of twenty-four to forty-eight hours there is acquired an insusceptibility to concentrations ten and more times as great as that in which growth of the untreated culture is inhibited. In our experiments, resistance developed whether the organisms were continuously or intermittently exposed to optochin.

Various experimental methods were tried to determine whether pneumococci can become insusceptible to the germicidal action of quinin upon prolonged exposure to a salt of this alkaloid. The experimental periods ranged from ten to thirteen days. In one series of experiments, 10 per cent of the cultures eventually grew in the highest dilution in which the control culture failed to grow. In no other series could even this extent of resistance be demonstrated. Against quinin, therefore, pneumococci do not show a similar tendency to the development of resistance.

(Discussion by Dr. C. W. Jungeblut, Albany.) I would like to know whether any tests were done to determine the cultural characteristics of the fast strains, and also whether the specificity of this fastness was determined.

(Discussion by Dr. Charles Weiss.) Optochin, as you know, is a synthetic salt of quinin, also known as ethyl hydrocuprein hydrochloride. Dr. Kolmer and I worked with this as well as with a number of other quinin compounds, and it is interesting to note that there is a marked difference in the activity of synthetic and natural compounds. One of the interesting things we observed which is very striking is that the intracutaneous test which can be used to demonstrate hypersensitiveness to quinin salts (first shown by Boerner) behaves differently when using the synthetic salt and when using the natural salt. I used this skin test on Dr. Boerner, who is extremely hypersensitive to quinin,

employing a series of about fourteen different salts, beginning with quinin alkaloid all the way up to the synthetic quinin. I found that although Dr. Boerner reacted to the natural quinin salts he did not react to the synthetic quinin, optochin.

(Dr. Meyer Solis-Cohen, closing.) I do not think the tests mentioned by Dr. Weiss were made.

THE EFFECT OF INTRAVENOUS INJECTIONS OF INDIA INK IN THE IMMUNIZED ANIMAL. Claus W. Jungeblut and (by invitation) Joseph A. Berlot, Albany.

Abstract. Massive doses of India ink injected intravenously into guinea-pigs before immunization with toxin-antitoxin mixture caused a delay in the appearance of diphtheria antitoxin for about one week. Intravenous injections of India ink into guinea-pigs were followed by a transitory drop of the complement titer. Reduction tests (methylene blue and nitro-anthraquinone) showed the respiration of the cells of the liver and spleen of the same animals to be markedly impaired for the first day. India ink injected intravenously in large doses into guinea-pigs before active sensitization caused a more or less marked decrease in sensitiveness to the reinjection of the antigen, while, when injected before passive sensitization, it did not interfere with the occurrence of anaphylactic shock. The precipitin titer of blocked sensitized rabbits was somewhat lower than that of controls. The titer of anaphylactic antibodies differed only in one case.

(Discussion by Dr. F. P. Gay, New York.) This last chart is particularly interesting in connection with antibody formation. It fills a gap which must exist between the apparently diverse results of Lewis who thought that blocking, or rather tuberculosis in his case, stimulated antibody formation and those observations, including our own, which indicate that antibody formation may be prevented with trypan blue. It is merely a relative difference. A small amount of colloidal blocking substances will stimulate antibody formation, and a larger amount may prevent it entirely. I think this last experiment is a very clear representation of that point.

(Dr. C. W. Jungeblut, closing.) I agree with Dr. Gay that the discrepancies reported in the literature may be fully explained by the fact that different authors used different experimental animals, different substances, different doses and also determined the antibody titer at different times during the immunization.

STUDIES ON IMMUNITY TO TUBERCULOSIS. Henry Stuart Willis, Baltimore.

Abstract. The most acceptable view of immunity to tuberculosis is that this phenomenon is very closely related to hypersensitiveness to tuberculin. We know that hypersensitiveness as shown by the tuberculin reaction in human beings may fluctuate considerably during normal life, becoming very slight or completely absent at times and developing again at other times. The question of whether immunity to tuberculosis fluctuates in this manner is an engaging one and one the answer to which has been sought by the following experiment.

The plan of the experiment was to study tuberculin sensitiveness of the skin of guinea-pigs for a relatively long time after infection and to compare the state of allergy as shown by skin reactivity with the degree of immunity to reinfection. To this end, guinea-pigs were first inoculated with a strain of tubercle bacillus of low virulence (R1) and at trimonthly intervals, beginning one year after in-

fection, a certain number of them were given intracutaneous tests with tuberculin and were, a few days later together with normal animals, inoculated subcutaneously with an emulsion of virulent tubercle bacilli.

Normally, the reaction to tuberculin injected into the skin of hypersensitive guinea-pigs is an inflammation, characterized by redness, induration and frequently hemorrhage and necrosis, the height being reached within twenty-four to forty-eight hours of the injection. The animals in this experiment that were tested with tuberculin one year after the initial, sensitizing inoculation (R1) were found in general to give very vigorous reactions. Animals allowed to go eighteen months before test were also found to react but with less vigor. At twenty-one months the reaction in those tested was still less marked and usually by twenty-four months no reaction could be elicited with the ordinary doses of tuberculin. Some of these latter animals, however, gave reactions with larger doses of tuberculin.

After each group of animals were tested with tuberculin they were inoculated with virulent tubercle bacilli (H37). Those receiving the second inoculation, two years after the initial infection, although they had lost their skin sensitiveness, still had a high degree of immunity — as high as had those reinfected at 12, 15, 18 or 21 months after the sensitizing infection or as had animals reinfected only two or three months after initial infection.

It was interesting to note that, although the skin-sensitiveness of the animals two years after the initial infection was in abeyance and could not be elicited with the dose of tuberculin ordinarily used, yet this sensitiveness was very quickly restored after reinfection, for the animals four or five days after reinfection exhibited very marked reactions to tuberculin.

The question arises as to whether immunity and hypersensitiveness do actually coexist *at all times* and whether fluctuation in hypersensitiveness necessarily indicates fluctuation in immunity. It appears that the degree of immunity of infected animals does not decline quantitatively in proportion to the decline of allergy *as we have been accustomed to test these phenomena.* But the failure to give skin reactions is perhaps not to be considered as absolute proof of the complete absence of hypersensitiveness. Is the immune state one of delicate balance, which, though not always demonstrable to test, may be present and may very quickly be raised to a high degree? There may be an immunity of minimal stationary degree which is present so long as infection exists or so long as any hypersensitiveness exists, even in slightest degree.

(Discussion by Dr: S. A. Petroff, Saranac Lake.) I was very much interested in Dr. Willis' paper. We have been carrying on the same type of experiment for the last four or five years, using, however, dead tubercle bacilli for our sensitization and not the strain of culture used by Dr. Willis, which is RI, a culture of low virulence. We have found that skin hypersensitiveness produced in guinea-pigs with dead tubercle bacilli is not of the transitory type. A fairly good hypersensitiveness lasting for over a year can be set up in a perfectly normal animal with dead organisms.

Another thing that interested me considerably in Dr. Willis' paper was the relationship noticed between skin hypersensitiveness and immunity in his animals. As I understand from what he tells us, guinea-pigs inoculated with RI organisms become skin hypersensitive, which state may last a year or two, after which the animals become skin negative and at this stage, although not hypersensitive, a degree of resistance could be demonstrated.

Our experience has been somewhat similar to that of Dr. Willis. In a large

series of experiments we have noticed that animals treated with dead tubercle and having lost their hypersensitive state, still possess some degree of resistance.

I am inclined to believe that we may be wrong in saying skin hypersensitiveness means resistance and that in all probability it has nothing to do with the basic mechanism of immunity.

(Discussion by Dr. R. R. Mellon, Rochester, N. Y.) I would like to ask a question about the time of the development of the immunity processes in relation to the time of development of hypersensitivity. Is it definitely known whether the time of the immunity development is synchronous with the allergic development or does it come sometime later?

(Discussion by Dr. L. Dienes.) The close relation between hypersensitivity and immunity was questioned by Lowenstein on the ground of the experience with bovo-vaccination according to Behring. After vaccination it was found that the majority of the resistant cattle did not react to the subcutaneous administration of tuberculin. Also in recent experiments of Calmette on cattle, with the B. C. G. bacilli, it was found that the hypersensitiveness, determined in this case with the intracutaneous test, disappears four to twelve months after vaccination, whereas the animals tested eighteen months after the vaccination were found resistant.

In forming any conclusion from this observation great caution is necessary because we do not exactly understand what is really indicated by the skin test, and on the other hand it is known that the result of the test is influenced by the technic and by the preparations used for it, and it might be that a quite considerable grade of allergy remains unnoticed in our test. So we do not think that there is sufficient ground for denying the connection between the hypersensitiveness and resistance, which is supported by good evidence, yet a certain amount of doubt is necessary.

(Dr. H. S. Willis, Baltimore, closing.) It is quite true that the dosage is of great importance. In these experiments all the animals received a constant dose at the time of inoculation, and it was the examination of these animals which had received constant doses, at intervals afterward that the work was based upon. As to whether immunity and hypersensitiveness have their inception at the same time after the preliminary inoculation, I cannot speak with absolute surety. I know that, depending upon the size of the dose, the hypersensitiveness may develop in 6, 7, 8, 9 or 10 days; or with very small doses not until two or three weeks. I know that surely within a few days of the development of the skin sensitiveness the animal is immune. It seems highly probable that they have their inception at the same time. They certainly develop at about the same time and coexist as complements of each other.

New Methods for Study of the Serum Sensitization of the Acid-fast Bacteria. Stuart Mudd and (by invitation) Emily B. H. Mudd, Philadelphia.

Abstract. The interfacial tension method (J. Exper. Med., 1924, xl, 633, 647; 1926, xliii, 127) has been used to study the sensitization of acid-fast bacteria. The bacteria are suspended in salt solution and observed with the dark-field microscope at the oil-water interface of a two-phase film. Before sensitization the bacteria are extremely miscible with the oil (tricaprylin, Kahlbaum). Clumps are dispersed explosively by the interfacial tensions and the bacteria are shot violently into the oil. After sensitization with high concentrations of normal

serum or high or lower concentrations of homologous immune serum the bacteria are no longer oil-miscible; they are stable in the oil-water interface; clumps are much more coherent and are markedly resistant to wetting by the oil. This alteration of the bacterial surface by immune serums is specific.

Agglutination tests as ordinarily conducted are notoriously unreliable with the acid-fast bacteria, but may be made more dependable by a simple modification. After the macroscopic agglutination readings have been made in the usual way the tubes are centrifuged. The supernatant fluid is poured off and a few drops of salt solution are added to the sediment in each tube. The tubes are arranged in a rack and shaken uniformly until the control shows an even suspension. The organisms which have been treated with the higher concentrations of serum resuspend in flocculi whether or not they showed agglutination by the ordinary procedure. The size and coherence of the flocculi increases up to the highest serum concentrations even where there was a prezone by the usual method. The interface reaction similarly has shown sensitization of the washed bacilli to be maximal after treatment with serums of maximal concentrations, and the interface reaction is positive with inagglutinable strains. The agglutination prezone and the inagglutinability of certain strains are thus due to inhibition of clumping and not to a failure to bind agglutinins. Agglutinins are bound, but something prevents the bacteria from clumping until they are forcibly brought together in the bottom of the centrifuge tube.

The interface and the resuspension reactions have both been found to be more reliable detectors of the binding of antibodies by the acid-fast bacteria than the ordinary agglutination procedure.

The immune serums used in this study were kindly furnished us by Dr. J. Fürth and Dr. J. D. Aronson.

(Discussion by Dr. H. G. Wells, Chicago.) I would like to ask Dr. Mudd if he considers the absorption of the desensitized bacteria as evidence of the protein character of antibodies. It seemed to be readily interpreted as evidence in favor of that point of view.

(Discussion by Dr. F. M. Huntoon, Glenolden.) I would like to ask a question along the same line. Does Dr. Mudd consider the entire surface of the bacteria to be coated with this material derived from immune serum?

(Discussion by Dr. L. Dienes.) Did you examine the influence of a tubercle bacillus immune serum which had no affinity for the lipoid substances of the bacteria? Such serum can be obtained by the immunization of rabbits with a watery extract of the bacterium. Dr. Freund has found the isoelectric point of tubercle bacillus to be near to the isoelectric point of the protein substances of the bacteria, and to be quite different from the isoelectric point of the lipoids of the bacteria. So, in the coating of the bacterium also, the protein substances seem to play a certain rôle.

(Discussion by Dr. R. R. Mellon, Rochester, N. Y.). I would like to ask Dr. Mudd whether the fact that the antibody being aqueous soluble, and combining presumably with a similar material in the wall of the organism, would be any indication that the surface of the organism itself is really a mosaic of acid-fast material and aqueous material, and not a solid acid-fast surface?

(Dr. Stuart Mudd, Philadelphia, closing.) I think Dr. Wells has very happily expressed the matter. It is an item of evidence which taken with other evidence tends to make one feel that the antibodies probably are protein. It is not conclusive by itself. We must study this interface reaction with a lot of purified chemical substance. We have studied carbohydrate in the form of

shreds of filter paper and of cotton and starch grains. These are the least oil-miscible substances we have found. They stay away over on the water side of the interface. All non-acid-fast bacteria studied are stable in the interface. Oxyhemoglobin crystals and the protein film on oil droplets in milk are stable in the interface; as soon as the film is torn off by the interfacial stresses the milk oil droplet explodes into and mixes with the oil phase. So far as the evidence goes then, this stability in the interface shown by strongly sensitized bacteria is characteristic of protein and not characteristic of the small number of other chemical categories we have studied. But I feel that the number we have examined is too small to allow of any final generalization.

The interface reaction shows that specific agglutination may occur when only a small fraction of the bacterial surface has been coated with antibody. Whether or not the whole surface is coated by the strongest serums, I cannot say.

The work of Dr. Freund I consider pretty conclusive evidence that there is a certain amount of protein in the tubercle bacillus surface. We found a sharp isoelectric point on the acid side of which the bacteria became positively charged. There is no reason to expect a sharp isoelectric point either with the known lipoids or carbohydrates. Lysolecithin and lysocephalin have broad isoelectric ranges, but no sharp isoelectric point. There must be some protein in the tubercle bacillus surface, but I do not in the least agree with Dr. Freund that the surface is all protein.

As regards Dr. Mellon's question, I do not feel that I have any evidence as to what the chemical receptor on the tubercle bacillus surface is. The result of the combination with antibody is the covering up of the surface lipin, but whether the receptor is a part of the lipin radicle or protein or carbohydrate I see no way at present of deciding.

The Chemistry of the Coagulation of the Blood. Frank Maltaner and (by invitation) Elizabeth Maltaner, Albany.

Abstract. Certain observations have been made which suggest that the coagulation of the blood is a true example of chemical catalysis and does not involve enzymes. This catalytic reaction seems to have a broad significance, lending support to the speculations of Nolf that the coagulation process is of fundamental importance in the nutrition of the tissues and in the immune and anaphylactic phenomena.

The serozym solutions of Bordet and Delange, supposedly free from fibrinogen and owing their activity to proferment, were shown, when sufficiently concentrated, to contain fibrinogen. The activity of these solutions was apparently due to the fibrin formed as the result of the reaction of this fibrinogen with calcium and cytozym.

The phosphate plasma of these authors was found to owe its stability to the decomposition of some of the gelatinous tricalcium phosphate used in its preparation, instead of to the adsorption of proferment as they believed. This decomposition consisted in the reaction of the tricalcium phosphate with the sodium chloride of the plasma to produce free alkali and alkali phosphate. The addition of these substances to oxalated plasma conferred upon it all the properties of phosphate plasma.

It was observed that calcium did not function as an activator of a proferment but in reality reacted with the lipoidic constituent essential to blood coagulation to form a relatively insoluble calcium-lipoid compound and liberate acid. It

was further shown that not only fibrinogen but all the proteins of plasma when slightly acidified would combine with the active lipoid constituent to form a lipoid-protein complex which would precipitate from solution. When equivalent amounts of lipoid and protein were present the supernatant fluid after precipitation was found to be entirely free from both protein and lipoid.

While calcium in reacting with the lipoidic constituent, in the coagulation of the blood, would not produce sufficient acid to bring about the massive mutual precipitation of the lipoid and protein present, it would initiate a catalytic reaction which under the influence of contact could rapidly produce such a lipoid-protein complex possessing the character of fibrin.

In the coagulation of the blood, the ionized calcium salt initiates the reaction pictured in equation No. 1.

$$LO_2P\begin{array}{l}\diagup OH\\ \diagdown OH\end{array} + CaCl_2 = LO_2P\begin{array}{c}\diagup O \diagdown\\ \diagdown O \diagup\end{array}Ca + 2\,HCl.$$

The acid that is liberated reacts with the fibrinogen, with which the blood or plasma is saturated, before it can diffuse from the surface of the precipitated lipoid salt to form fibrinogen chloride as pictured in equation No. 2.

$$\begin{array}{r}HOOC\diagdown\\ HOOC\diagup\end{array}R\begin{array}{l}\diagup NH_2OH\\ \diagdown NH_2OH\end{array} + 2\,HCl = \begin{array}{r}HOOC\diagdown\\ HOOC\diagup\end{array}R\begin{array}{l}\diagup NH_2Cl\\ \diagdown NH_2Cl\end{array} + 2\,H_2O$$

This acid fibrinogen being formed in direct contact with the calcium-lipoid compound reacts with the latter to produce a lipoid-protein complex, which is fibrin, and sets free calcium chloride as illustrated in equation No. 3.

$$\begin{array}{r}HOOC\diagdown\\ HOOC\diagup\end{array}R\begin{array}{l}\diagup NH_2Cl\\ \diagdown NH_2Cl\end{array} + LO_2P\begin{array}{c}\diagup O \diagdown\\ \diagdown O \diagup\end{array}Ca$$

$$= \begin{array}{r}HOOC\diagdown\\ HOOC\diagup\end{array}R\begin{array}{c}\diagup NH_2O\diagdown\\ \diagdown NH_2O\diagup\end{array}PO_2L + CaCl_2$$

The liberated calcium chloride then reacts with more lipoid and the reaction proceeds to an equlibrium.

These reactions represent a true example of a chemical catalysis in which calcium chloride functions as the catalyzer, under the activating influence of contact. It is suggested that this catalytic reaction explains the mechanism of clot formation.

The lipoid compound obtained by precipitation with calcium chloride in ammoniacal solution possessed a percentage composition similar to that of cephalin containing one calcium atom. It was devoid of coagulating activity but treatment with dilute hydrochloric acid removed the combined calcium and left a compound soluble in alcohol and acetone and possessing the active coagulating function. Reprecipitation of the calcium salt from this material followed by treatment with dilute hydrochloric acid and resolution in alcohol did not affect the coagulating function of the material. The second calcium-lipoid compound, however, was found to be free from nitrogen and possessed a percentage composition equivalent to that of cephalin in which the amino-ethyl-alcohol radical and a hydrogen of the phosphate group were replaced by a calcium atom.

(Discussion by Dr. V. C. Jacobson, Albany.) I should like to ask Mr. Maltaner how, on the basis of his theory of coagulation, he would explain the lengthening of coagulation time following intravenous injections of peptones and foreign proteins.

(Discussion by Dr. H. G. Wells, Chicago.) I should like to ask whether the derivative of the cephalin in Formula 3 when added to fibrinogen in the absence of calcium produces fibrin, as I suppose must be the case according to the theory. I should also like to ask how, on the basis of this theory, the author would explain the common observation of the formation of fibrin when simply a foreign body not active chemically, for instance gold or platinum wire, comes in contact with the circulating blood.

(Discussion by Dr. G. H. A. Clowes, Indianapolis.) I am strongly in favor of this point of view. Ten or twelve years ago I did a lot of work in this field and I regret that I did not put it through as conclusively as this has been done. I am quite satisfied about that calcium-lipoid combination and I would like to ask whether that preparation is found to be heat resistant. I had a substance of this type that would stand boiling half an hour with little loss of activity. The whole picture that we have of lipoid-protein combination in the cell would fit in with this idea and the mere fact of using a needle or anything of that sort and introducing it into a vessel, leading to clotting, would be in harmony with that idea.

(Mr. Frank Maltaner, Albany.) In answer to the question in regard to the introduction of a needle, it is well known that the lipoid substance is contained in mammalian blood in the platelets and contact or the contact with a strange surface attracts the platelets to the strange surface and liberates there the lipoid substance. The calcium and fibrinogen are always present and the reaction is instigated at that surface.

This compound in Formula 3 when added to fibrinogen or when added to plasma — pure fibrinogen probably never has been had — will produce a precipitate. A clot is only formed when the catalytic process takes place.

The matter of heat resistance — the lipoid substance has been shown by all of the modern investigators of the coagulation question to be heat resistant.

(Discussion by Dr. G. H. A. Clowes, Indianapolis.) I do not mean the lipoid substance. I mean the thrombin substance that does the whole thing — the calcium-lipoid complex ready to act.

(Mr. Frank Maltaner, Albany, closing.) That complex — that is, the calcium-lipoid complex — is inactive, has no coagulative function. The thrombin, however, is not heat resistant.

(Dr. G. H. A. Clowes.) Yes, it is.

Bacterial Synergism. W. L. Holman, Toronto.

Abstract. The term synergism as applied to bacteriology defines the phenomena of the coöperative action of two or more bacteria which results in the formation of products not formed by the individual bacteria growing alone. The analogous phenomena of the suppression of metabolic products should also be grouped under this term since the products thus formed are also different.

Since 1912 I have been demonstrating to the students in bacteriology the gas producers when these are grown together in a medium containing carbohydrates or similar bodies unfermented by the gas producer. For example *B. paratyphosus* with a lactose fermenting streptococcus gives acid and gas in lactose

broth. Many other combinations were tried and the importance of the results was emphasized in tests for *B. coli;* in picking colonies of *B. paratyphosus* from Endo plates; and in the probable explanation of many altered fermentation tests with the colon-typhoid bacteria where one of the combinations may have died out between the tests. Theobald Smith and D. E. Smith in 1920 reported on the inhibitory action of paratyphoid as distinguished from the hog cholera bacilli on the fermentation of lactose by *B. coli.* Sears and Putnam in 1923 studied the same type of combinations as I had been using and obtained the same results. Castellani, 1925, also reported similar findings.

The present report covers attempts to learn more about the intimate metabolism of the bacteria and considers some of the factors concerned. Many combinations grown in mediums containing substances not attacked by the gas-former result in gas production if the second bacterium is capable of splitting to acid the substance used. The gas-former must also be capable of forming gas from glucose.

B. communis (non-saccharose fermenter) and *Streptococcus fecalis* were used in the more detailed study in plain extract broth with 1 per cent saccharose.

The two bacteria may be growing together and fail to give gas if the relative numbers of each bacterium are not suitable or if the hydrogen ion concentration does not favor gas production. All grades of gas production can also be obtained by varying the numbers of each organism in the seedings and the time of the addition of the second bacterium. This was shown to be partly due to differences of the two organisms in their rates of growth, the *B. coli* reaching its maximum in about half the time required for the streptococcus, and further to the differences in the pH value at different periods. The two bacteria must be living in close association. Filtrates of either or both organisms did not give gas when seeded with the other organism. When grown in the above medium in U tubes closed at one end and partly filled with sand there was no gas formed until both bacteria had penetrated the sand.

There is less carbon dioxide in the gas produced from saccharose by the combination than that formed by *B. coli* alone from glucose, the synergistic ratio being $\frac{H}{CO_2} = \frac{4}{1}$, that of the *B. coli* alone $\frac{H}{CO_2} = \frac{1.5}{1}$. This would suggest that part of the CO_2 was retained in the medium as carbonates from the action of the *B. coli* on the organic acids. Ammonium lactate in broth is acted upon by *B. coli* with alkali production and added to dextrose broth lessens the amount of acid and gas produced by this organism.

There are thus two opposite processes going on and the question of whether gas will be produced or not depends on which process is more active at the time in the metabolic life of the bacterium. It is to be emphasized that the above conditions may not infrequently occur and many errors in interpreting the results of fermentation tests can be thus explained. It is of importance in water work, isolations from stools and in general studies where fermentation tests are employed. The careful study of these synergistic reactions will lead to a better understanding of the metabolism of bacteria.

(No discussion.)

A Pathogenic Bacillus Resembling the Diphtheria Bacillus. Ruth Gilbert and (by invitation) F. Constance Stewart, Albany.

Abstract. A pathogenic organism resembling the diphtheria bacillus and similar to that described by Parker has been isolated from twenty-five throat cultures

received at this laboratory. In young cultures the organisms resemble diphtheria bacilli, polar bodies being readily demonstrated, while in older cultures coccoid forms predominate. The cultural characteristics are similar to those of diphtheria bacilli except that gelatin is liquefied in six to seven days and nitrates are not reduced.

Filtrates of broth cultures were found to be toxic and a horse was immunized with them. After thirteen months of immunization, 1/150,000 cc. of its serum neutralized 0.01 cc. of the toxic filtrate when injected intracutaneously in rabbits. Before immunization the serum of the horse contained less than 1/500 unit of diphtheria antitoxin per cc. but after immunization as much as thirty units were present at one time. One-hundredth cc. of the toxic filtrate was found generally to be neutralized by 2.5 units of diphtheria antitoxin. These results would indicate that the organism has some immunologic relation to the diphtheria bacillus.

(Discussion by Dr. M. A. Goldzieher, Budapest.) May I ask whether there was any difference in the morphology of the bacillus with Gram stain or with any other stain, for instance methylene blue? I have studied the bacillus of inguinal granuloma, the morphology of which is very similar to this one. This bacillus took on a coccoid form with the Gram stain but if stained with methylene blue without using alcohol or any other fat solvent substance then the bacillus form was still visible. I therefore conclude that there was a fatty degeneration in the body of the microörganism and this fatty substance which I think is a fatty acid was dissolved by absolute alcohol or other fat solvent substances which I used. May I ask if such observations were made in this case?

(Miss F. C. Stewart, Albany, closing.) The organisms we studied were gram-positive and showed the same changes in morphology at the different incubation periods with the Gram stain as was noted with Loeffler's alkaline methylene blue stain. We did not use an aqueous methylene blue.

THE PASSAGE OF BACTERIA FROM THE PERITONEAL CAVITY INTO CAPILLARIES AND LYMPHATICS IN COLON BACILLUS PERITONITIS. Bernhard Steinberg (by invitation) and Harry Goldblatt, Cleveland.

Abstract. Simultaneous cultures of blood (femoral artery) and lymph (thoracic duct) were taken after the intraperitoneal injections of (*a*) *B. coli* suspended in physiological saline and (*b*) *B. coli* suspended in gum tragacanth.

In the experiments with saline suspensions, the bacteria appeared in lymph and blood, a little earlier in the former. Estimation of the number of bacteria showed a great preponderance in lymph.

In the experiments with gum tragacanth suspensions, bacteria appeared in lymph but not in blood. The number of bacteria in this lymph was very much smaller than in the lymph after the injections of saline suspensions.

Dogs injected intraperitoneally with *B. coli* and gum tragacanth developed a hemorrhagic sero-fibrinous peritonitis and died. Dogs injected intraperitoneally with *B. coli* suspensions in saline showed a slight hyperemia of the peritoneum, developed a bacteremia but survived.

It is suggested that the fatal results after injection of gum tragacanth suspensions may be due to the elaboration of the *B. coli* toxin in the peritoneal cavity. This phase of the work is being continued.

(Discussion by Dr. Stuart Mudd, Philadelphia.) Did you attempt to see if the gum tragacanth opsonized the bacteria?

(Dr. B. Steinberg, Cleveland, closing.) No.

UNDULANT FEVER IN MAN ASSOCIATED WITH BACTERIA INDISTINGUISHABLE FROM BRUCELLA ABORTUS. V. A. Moore and (by invitation) C. M. Carpenter, Ithaca.

Abstract. The purpose of this paper is to describe a few cases of undulant fever in man caused apparently by infection with the organism of the Bang abortion disease and to point out the similarity that exists between it and Malta fever. The relation of *Brucella melitensis* to the organism found in the cases of undulant fever is like that between *Br. melitensis*, found occasionally in goat's milk, and *Br. abortus*, sometimes found in milk of cows that have aborted. A brief review of cases of human infection with this organism have already been published by Keefer, DeKorte, Orpen and others and the results of the tests for antibodies of *Br. abortus* in human blood. A brief review of the cases is supplemented by six cases of undulant fever in which *Br. abortus* was isolated from the blood. Abortion was produced in pregnant heifers with cultures isolated from four of the cases. The inoculation produced a more severe reaction in cattle than usually follows the injection with *Br. abortus* isolated from infected cows. There are no cases of abortion reported in the human species in which this organism has been established as the cause. *Br. abortus* was isolated from cows' milk in 1911 and 1912 by Schroeder and Cotton and Smith and Fabyan, and since that time it has been found frequently in the milk of herds in which abortion exists. The source of infection in the human cases reported is not determined. It is known that two of them drank freely of raw milk from herds where abortion in the cattle had occurred and in the milk of which the organism was found. A study is being made of the bacteria in market milk in reference to the prevalence of this organism and the virulence of the different strains. As a precaution pasteurization of milk is recommended.

(This paper discussed with the next paper.)

A COMPARISON OF STRAINS OF BRUCELLA ABORTUS ISOLATED FROM MAN WITH THOSE FROM CATTLE. C. M. Carpenter (by invitation), Ithaca.

Abstract. The author has isolated five strains of *Brucella abortus* from the blood and urine of men suffering from undulant fever. The cultures were isolated by sealing the tubes with wax and subjecting the cultures to an atmosphere where 15 per cent of the air was replaced by 10 per cent CO_2. Cultures were positively identified as *Brucella abortus* by the agglutinin absorption test. The cultures isolated from man were compared with those from cattle with regard to the following characters: atmospheric requirements, serologic relationships, pathogenicity for guinea-pigs, their effect upon pregnant cattle when injected intravenously, and their ability to establish themselves in the udders of cattle.

The results of these studies disclosed the following facts. The first generation of the human strains was always very difficult to grow, but the second and following generations grew well upon unsealed tubes of nutrient agar. Certain bovine strains which the author has studied have shown this same characteristic while others have, for longer or shorter periods of time, required an increased amount of CO_2 and some enriching substance in, or added to, the medium, such as sterile blood or serum. The cultures isolated from men have been extremely virulent for guinea-pigs, but not more virulent than certain strains of *Brucella abortus* isolated from bovine sources. Four of the five human strains have produced abortion in pregnant heifers when injected intravenously. The fifth strain was injected only very recently, but, judging from the symptoms the animals is showing at present, she will abort. The organism has been recovered

from the fetuses, placentas and milk of the four cows which have aborted. One strain of *Brucella abortus* of human origin established itself in the udder of a heifer and remained there for six and one-half months when the animal was destroyed. The cultures isolated from man were more toxic for the pregnant heifers than were bovine strains and produced abortion in a shorter period of time.

(Discussion by Dr. James Ewing, New York.) Will Dr. Moore kindly state if there was any involvement of the lymphatic system in this infection in man or animal? Has the condition any resemblance to the so-called glandular fever? Was the structure of the glandular lesion in cattle that of suppurative inflammation, or of a lower type of inflammatory reaction?

(Dr. V. A. Moore, Ithaca.) We find in the calf some enlargement in the presence of the organism in the lymph glands. It is quite possible that glandular fever may be due to this organism but it has not been considered in our studies and we have not carried the work far enough to draw conclusions on this point. In regard to the udder changes, we have not finished our studies on that subject. Most of the cases of abortion in cattle do not come to postmortem and we do not have a chance to study the udders histologically. We are undertaking now a study not only of the changes in the udder but also of the cellular content of the milk which may be somewhat different from that of ordinary pyogenic infections.

(Discussion by Mr. J. G. Olson, Indianapolis.) There are a number of well known foci in this country, particularly in Texas, Arizona, New Mexico and Utah from which Malta fever might be disseminated. In the spring of 1921 we were able to obtain histories of something more than fifty cases of definite Malta fever which had occurred in human beings in Utah, over a period of about fifteen years, with six deaths. This mortality was unusually high. There are several foci in Utah where goat's milk is produced and cheese is made from the unpasteurized milk. The cheese is sent to New Orleans and other places. Even as tularemia is moving eastward so from such foci of infection we may find Malta fever spreading over the country and this work serves to call to mind the fact that undulant fever should be followed up bacteriologically very carefully and undoubtedly in the near future many more cases will come to our attention.

(Discussion by Major James Coupal, Washington.) I would like to know if any of the authors' cases occurred in the region of Plattsburg, N. Y., or Fort Ethan Allen, Vermont.

(Dr. V. A. Moore, Ithaca, closing.) There were no cases from Plattsburg or Fort Ethan Allen. Goats do suffer from abortion the same as cattle. I have a verbal report of one instance of this trouble in the Southwest (Arizona) where 50 per cent of the pregnant goats in a herd aborted. The cause was not determined but it is presumable that it was due to one of the Brucella as the history indicated that it ran a course similar to the Bang abortion disease in cattle.

Epidemic Hiccup: Animal Experiments and Microscopic Lesions of Brain and Cord in One Case. E. C. Rosenow and (by invitation) Harry Parker, Rochester, Minn.

Abstract. The patient, a man 68 years of age, while in the hospital prior to a contemplated prostatectomy for hypertrophy of the prostate at a time when a mild epidemic of hiccup was occurring, developed fever, inability to talk above

a whisper, difficulty in swallowing, intermittent hiccup, vomiting, restlessness, marked generalized weakness and difficulty in breathing with choking and syncopal attacks, in one of which he died two days after onset of symptoms.

Neurologic examination by Dr. Harry Parker revealed loss of the left corneal reflex, a crossed anesthesia of the left side of the face and the right side of the body and the findings of a left vagoglosso-pharyngeal hypoglossal paralysis indicating lesions in the left side of the upper medulla, involving the nuclei of the ninth, tenth, eleventh and twelfth cranial nerves and the spinal root of the fifth nerve.

Necropsy revealed chronic sinusitis, nasal polypi, hypertrophy of the prostate, marked arteriosclerosis, especially of the cerebral vessels, and retrograde thrombosis of the left posterior inferior cerebellar and vertebral arteries. No gross lesions of brain and cord were found.

On account of the intermittent attacks of hiccup and the occurrence of a mild epidemic of singultus at the time, it was thought that possibly the symptoms might be attributable to the streptococcus which has been identified with epidemic hiccup and encephalitis. Accordingly, cultures and animal inoculations were made with swabbings from the nasopharynx, from the catheterized urine during life, and from the blood and urine after death. The streptococcus identical to the one isolated in epidemic hiccup and which produced spasms of the diaphragm or other muscles, associated with bulbar symptoms, in inoculated animals, was obtained from nasopharynx, urine and blood. It was isolated in pure culture from the brain and medulla and demonstrated in the lesions in the animals that developed characteristic symptoms.

Microscopic sections of the brain and cord of the patient revealed two poorly staining areas of infarction on the left side of the medulla, the larger in the posterior and the smaller in the anterior aspect in the region of the olive. These were largest at about the level of the vagus nucleus and rapidly diminished in size above and below this level. In these areas there was found thrombosis of vessels, light staining and degeneration of nerve cells, leucocytic and round cell infiltration and slight perivascular infiltration by leucocytes and round cells. No noteworthy lesions were found in sections taken from various parts of the brain, pons and spinal cord. Gram-positive diplococci, singly, in groups and sometimes in chains of two, were found within and adjacent to the lesions in the medulla and within one of the thrombosed blood vessels. No bacteria were found in sections remote from lesions.

(Discussion by Dr. James Ewing, New York.) How long after the operation did the patient die? How long after death was the autopsy performed?

(Dr. E. C. Rosenow, Rochester, Minn., closing.) The patient was not operated upon. He was merely in the hospital being prepared for prostatectomy. The autopsy was held two hours after death.

Microscopic Lesions of the Central Nervous System in Rabbits Injected with Streptococci from Encephalitis, Spasmodic Torticollis and Poliomyelitis. E. C. Rosenow and (by invitation) M. Balado, Rochester, Minn.

Abstract. The relationship between the symptoms and the lesions found in rabbits after injection of virulent material of epidemic encephalitis, is an object of discussion because spontaneous encephalitis of rabbits with its typical anatomic pathologic picture rarely gives rise to symptoms.

In an extended study of the effects of inoculation of the streptococcus isolated in encephalitis and allied conditions, marked discrepancies between symptoms and lesions were sometimes noted, especially as regards the late manifestations or so-called sequellae. With the object of establishing this point, we have examined serial sections of the brains of three rabbits that showed characteristic symptoms. One of these (R 1080) was injected 160 days previously with the streptococcus from the nasopharynx in a case of spasmodic *torticollis;* one (R 1174) eighty-four days before with a suspension of the streptococcus from poliomyelitis and the third, thirty-one days before with a green-producing streptococcus from milk and which produced encephalitis in rabbits.

Rabbit 1080. The head always tilted and rotated to the left to approximately an angle of 90 degrees. The right eye looked up, the left down. The left pupil was larger than the right. When the animal was held vertically in palm of hand, the abnormal position of the head increased; when held over the back with head downward, right side of body rotated to the anterior position and the head became completely rotated. The abnormal rotation was corrected when the animal was lifted by the ears.

Rabbit 1174. When quiet in cage, animal appeared normal. When prodded, it always went backward. When held upright in the palm of the hand, the head always was tilted to the left. The left fore extremity was extended and the right flexed. Hanging with the head down, the head was drawn to the left.

Rabbit 1213. Symptoms were similar but more marked than those in Rabbit 1080, but torsion of the head and body was to the right instead of to the left. Under excitement, horizontal nystagmus occurred. The pupils reacted slowly to light. The position of the body and head was not corrected when the animal was lifted by its ears.

The animals were all injected intracerebrally, well forward in the right frontal lobe and were killed with chloroform. No microscopic changes were found at necropsy. The sections were stained by Nisal's method, as modified by Leukossek and Jakob of Buenos Aires and examined for lesions throughout the brain substance, paying especial attention to their distribution, especially with regard to the nucleus of the vestibular nerve.

Widely disseminated foci of lymphocytic infiltration were found in Rabbits 1080 and 1174. These were most numerous in the cortex, in lesser numbers in the thalamus, and cerebral peduncles and only rarely occurred in the pons. They were bilateral and showed no relation between their location and the symptoms of the rabbits. The nerve cells, especially in the brain stem were normal in size and staining reaction. In Rabbit 1213, the one with the most marked postural symptoms, no foci of lymphocytic infiltration were detected and the nerve cells appeared normal throughout.

In four other rabbits with similar symptoms, search for lesions was made in frozen sections stained by the method of Spielmeyer, for myelin. No lesions were found of the vestibular nerve.

On the basis of these findings, we would conclude that with the methods employed, we cannot detect changes in nerve cells that explain the particular symptomatology in the rabbits examined and that the foci of lymphocytic infiltration (the typical histologic picture of spontaneous encephalitis in rabbits) have nothing to do with the symptoms induced by the injection of the streptococcus of epidemic encephalitis and allied conditions.

(No discussion.)

THE FILTERABILITY OF THE MOUSE SARCOMA AND CARCINOMA. M. J. Sittenfield, New York City.

Abstract. Gye's report, setting forth his conception of the etiology of cancer, aroused so much interest that it became most essential to confirm his experimental evidence. For this purpose mouse sarcoma No. 37 was used. The injection of the supernatant fluid from anaerobically incubated cultures of mouse sarcoma No. 37 yielded in our hands 32 per cent of tumor takes. The tumors appear on the average seventeen days after injection. Microscopically, they closely resemble the parent tumor. As a control to this experiment the possibility of cells remaining alive in the fluid was tested by inoculating the incubated tumor tissue itself. Out of sixty-three inoculations of washed tissue, there were but two takes, out of fifty-eight inoculations of unwashed tissue there were three takes. If our results are due to the presence of live cells it is difficult to reconcile the 32 per cent of takes from the supernatant fluid with the very small percentage of takes obtained from the inoculations of the incubated tumor tissue itself.

The acid test of ruling out the presence of cells in the incubated fluid was met by the filtration of the fluid through a Berkefeld filter "N," which had been tested against bacteria before and after filtration.

For the mouse carcinoma No. 63 we claim only two takes from the supernatant fluid, none from the filtrate. We are thus able to confirm Gye's experiments concerning the filterability of mouse sarcoma No. 37, which yielded in our series of experiments eight tumor takes out of 183 mice, or about 4 per cent of takes in the mice injected with the cell free filtrate of mouse sarcoma No. 37.

(Discussion by Dr. I. P. Lyon, Buffalo.) In this connection I recall some experiments that I saw reported in the Journal of Parasitology about a year or so ago in which some one had succeeded in filtering trypanosomes and securing successful takes from the filtrates by the material going through Berkefeld filters of some sort, I do not know the size. I was very much astonished to see that enough of the trypanosomes could go through in order to grow. It would seem it is possible that a cell, such as a trypanosome, possibly as large as a tumor cell, does send something through a Berkefeld filter which insures growth when placed in a suitable environment. Such a conception of a cell is beyond our ordinary ideas but that work on trypanosomes and this work referred to in regard to the filterability of tumors suggests that possibility. It seems to me it is quite as probable that something goes through from the tumor cell, which enables the cell to reproduce, as that it is a filterable virus (about which we know nothing) which goes through.

(Discussion by Dr. W. L. Holman, Toronto.) As shown in the paper to be read by title later, we tried a number of filter candles by testing the amount of fluid that went through in a given time. If we oiled them with vaseline or a mixture of paraffin oil and paraffin, they became very much more permeable to bacteria and less permeable to fluid. In other words, we did away to a great degree with the adsorption on the surface of the pores of the filter. This may be interesting in regard to the possibility of filtering substances adsorbed on the surfaces of the clean or unoiled filter.

(Discussion by Dr. Stuart Mudd, Philadelphia.) It is not a simple matter. It is very far from being simple. H. Rechhold published a formula by which the size of the Berkefeld or other filters could be estimated. Applying the formula in its original form we reach the conclusion that the diameter is about 0.4 micron.

As pointed out by Bigelow and Bartell (J. Am. Chem. Soc., 1909, xxxi, 1194) a factor in the denominator of Berkefeld's formula was ten times too big. That brings the actual intergranular diameters of clean Berkefeld filters up to about 4 microns. That is a pretty big space. The difference in the three types of filters (V, N and W) is not in the intergranular spaces but in the amount of coarse pores present. This very interesting report of Dr. Holman shows, as many of us have concluded from other evidence, that the pores of a clean filter were plenty big enough to let through bacteria. Indeed, Wolbach, using spirochetes whose filterability had been enhanced by long continued selection, was able to demonstrate them in the filtrate immediately after passage through a Berkefeld filter. Berkefeld filters appear to owe their tightness for bacteria (1) to the tortuosity of the channels through them; (2) to the fact that adsorption of protein or other matter from the filtering fluid quickly reduces the diameters of the intergranular spaces; adsorption is less if the filtering fluid is weakly alkaline than if it is acid —*i.e.*, on the alkaline side of their isoelectric points, the ampholytes present are negatively charged and hence do not so readily adhere to the electro-negative filter pore walls; (3) probably also adsorption of the bacteria themselves on the filter pore walls play a part; (4) if the filtration is continued for a considerable time a "cake" forms on the outside of the candle and is in itself a filter. No doubt other factors also influence the filtration process. Certainly we cannot conclude, as is so often done, that because things go through a Berkefeld filter they are below the dimensions of microscopic visibility.

(Discussion by Dr. R. R. Mellon, Rochester, N. Y.). I want to call attention to the fact in connection with trypanosomes passing through the filter, it is not a bad idea to remember that an increasingly large number of organisms are being found to have filterable stages in their life history. I recall that eight years ago we were repeatedly able to pass a filterable organism from the blood serum of the patient's blood through a filter. This organism grew then in the filtrate as a staphylococcus which would not go through the same candle, and yet we could repeatedly pass diluted specimens of this blood serum through this filter candle. It is claimed too that tubercle bacilli have a filterable stage.

(Dr. M. J. Sittenfield, closing.) It is not my intention at this time to discuss the presence of a microörganism in cancer. In our present report we make no claim that we are dealing with a microörganism in the filtrate. At any rate, it seems to me most improbable that cancer cells could have passed through a filter. It may be interesting to relate in this connection that for about two months and a half our attempts to get a tumor from filtrates were negative, until we refined our technic, eliminated chloroform or ether as an anesthetic, etc., and perfected the anaerobiosis. It was found that by protracted exposure of the culture fluid to the air during filtration we were not able to get a tumor, and only by revising our technic and excluding the air as much as possible during the time of filtration were we then able to get 4 per cent of tumors from the filtrate in the injected animals.

THE CAUSE OF DEATH IN CARCINOMA CASES. Margaret Warwick, St. Paul.

Abstract. This is a statistical study of 560 cases of carcinoma found in the 7900 necropsy reports on file in the Department of Pathology at the University of Minnesota. These cases were analyzed from the point of view of the major complications of the carcinoma, particularly in regard to the immediate cause of death. Of these, carcinoma cases of pancreas, gall bladder, esophagus,

larynx, pharynx, duodenum, and bladder, all showed death to be caused by major complications, and not the tumor growth alone. But the carcinoma alone caused death in 2 or 6 per cent of the cases involving prostate, 1 or 58 per cent of malignant melanomas, 8 or 53 per cent of ovary, 15 or 42 per cent of the lung, 3 or 7 per cent of rectum and sigmoid, 4 or 16 per cent of uterus, 15 or 39 per cent, and 25 or 14 per cent of the stomach. Bronchopneumonia was the most frequent cause of death in carcinoma of the esophagus, ovary, lung, pharynx, breast, and in malignant melanoma. Peritonitis was the most frequent cause in carcinoma of colon, rectum and sigmoid, bladder and stomach. Hydronephrosis was the most frequent cause in carcinoma of prostate and uterus. Obstructive jaundice was the most frequent in carcinoma of the pancreas and gall bladder.

(Discussion by Dr. Alfred Plaut, New York.) I would like to ask whether all the patients who died, died from cancer alone. Were they emaciated or were there some who showed a good amount of subcutaneous fat, for instance? I have seen cases where no direct cause of death could be found.

(Discussion by Dr. M. A. Goldzieher, Budapest.) I would like to call attention to one point which seems to have been disregarded. It is well known that the heart muscle in many cancer areas is subject to fatty degeneration with consequent dilatation of the heart, which may account directly for the death. I think that in these cases of "carcinoma only" quite a few were due to such heart failures. Anemia is a much more frequent cause of death in cancer than these statistics would show.

(Dr. M. Warwick, St. Paul, closing.) There seems to be no rhyme or reason about the emaciation. A great many of these patients were markedly emaciated and a great many were not, but those who died of carcinoma alone were not more emaciated than were the others, and I found that a few patients dying from carcinoma with metastases were well nourished.

As far as the condition of the heart muscle goes, I feel confident that a number probably died from heart failure. It is impossible to measure the exact degree of involvement of the heart muscle at the autopsy so I did not include that in the causes of death. However, many of them were listed as having dilatation of the ventricle or myocarditis and these probably died of heart failure. The same is true of anemia. I feel sure that many of these patients died of anemia but there is no way of determining this at the autopsy alone, and since we cannot always get full clinical notes I did not include anemia as a cause of death unless I knew definitely that it had been very pronounced during life.

A few of these carcinoma cases died an accidental death, and one was suicide.

Histological Picture and Prognosis in Carcinoma of Uterine Cervix.
Alfred Plaut, New York.

Abstract. Three different problems are presented: first, predicting the clinical course from the microscopic picture in any tumor; second, the same for one definite group of tumors; and third, regarding a certain kind of tumor in a certain organ. Further problems are grouped around the words "degree of malignancy" especially when established from a "cell type."

Among many cases which were studied 150 offered data of sufficient exactitude. An attempt was made to follow Martzloff's classification ("spinal cell, transitional cell, fat spindle cell"). The examination of the slides preceded

the study of the clinical course. We did not, however, find a definitely better expectancy of cure in the groups which are supposed to be less malignant. Only for the cases with hornification the surviving rate was higher. Spinal without hornification showed even lower survival rate than the "transitional" cases which are considered more malignant by Martzloff. No group of real "fat spindle cell" carcinoma could be established. Among nine cases in which the presence of fat spindle cells was conspicuous, four were living and well after three years. Thus a tumor containing many spindle cells does not mean necessarily an unfavorable course of the disease. The question of adenocarcinoma was omitted in this paper for lack of sufficient data. The distribution of our material according to the groups of Martzloff would be: 108 spinal, 22 transitional, 10 adenocarcinoma, 1 spindle cell carcinoma and 8 undefinable. A more differentiated grouping would be:

30 spinal with hornification
56 spinal without hornification
5 spinal and transitional
10 between spinal and transitional
7 transitional and spinal (some with horn)
22 transitional (including wide variations)
10 adenocarcinoma
1 spindle cell carinoma
8 undefinable

The borderline between spinal and transitional has been drawn differently by Martzloff and by us. This difference does not interfere with the comparison of results. Cases with five year cure and cases with death within one year showed the same distribution among the histologic groups. The cases with three and four years' cure had the same number of spinal that all cases had. The surviving rate did not show a higher figure for the spinal cases, but the same as for transitional. This lack of difference between his groups somehow has been stated by Martzloff himself. He says that tumors belonging to different groups grow through the uterine wall with the same speed; and he saw no cure after true broad ligament involvement. The consideration of all this together shifts the prognosis again to the clinician. In ten slides the histologic picture was very irregular, one belonged to a patient who lived for two and one-half years after onset of symptoms; all the others died within one year. In such striking cases histologic prognosis can be possible. No other single histologic feature was found in direct relation to the outcome and no group of such features either. It is the same with the results of clinical pathology.

Undifferentiated cells are considered to indicate a highly malignant tumor. Since they are on the other hand highly susceptible to radiation another difficulty is introduced into histologic prognosis.

The cellforms in cervical carcinoma are so manifold that grouping seems a hopeless task.

Conclusions: (1) We have no reliable basis for histologic prognosis in cervical carcinoma.

(2) The cellforms of cervical carcinoma do not admit of establishing well-defined groups.

(3) In speaking about malignancy the radiosensibility must be considered separately.

(4) The clinical classification is still the best aid in making a prognosis.

(Discussion by E. T. Bell, Minneapolis.) Our experience has been somewhat the same as that of Dr. Plaut. We cannot form an accurate idea as to the malignancy of the tumor on the histologic structure alone, except in the case of markedly undifferentiated tumors which are more malignant. The extent of the tumor at the time the treatment is instituted is of much more value in prognosis.

(Discussion by Dr. James Ewing, New York.) There is much value in emphasizing, in the minds of pathologists and surgeons, the great variation in the prognosis of cancers, and the considerable dependence of this prognosis upon structure.

Recently, I have been going over a series of mammary cancers from this point of view. All the relatively benign cases were picked out successfully and all the very malignant ones. There was, however, an intermediate group in which many errors were made in predicting the outcome on the structural type. It became evident that these errors would have been greatly reduced, if attention had been paid to the duration and extent of the disease, at the time of the operation. In this large group of cases, it seems to me unwise to attempt to establish a prognosis on structure alone.

I agree with Dr. Plaut that every case of cancer should be regarded as a special study, on its own merits.

(Discussion by Dr. F. B. Mallory, Boston.) I would like to bring up one point not yet mentioned. If you find one or two mitotic cells you know your tumor is growing. If a dozen, you know it is growing rapidly. That is the point on which you want to base your prognosis as to whether the case is going to end quickly or not.

(Dr. Alfred Plaut, New York, closing.) I must apologize to the members that I omitted certain figures. This material has been fixed after removal and one section may have been fixed shortly after and another one much later. The fixative was formalin. I could not find a direct relation between the number of mitotic figures, irregular or regular ones, and the outcome of the disease. I spent a good deal of time in doing this and I had to give it up. I spoke only about the carcinoma of the cervix. I do not know whether it will be possible to establish the kind of diagnosis in other kinds of cancer and in other organs.

The Solid Type of Osteitis Fibrosa. J. S. McCartney (by invitation), Minneapolis.

Abtsract. Of seventeen cases of osteitis fibrosa on record in the Department of Pathology, University of Minnesota, four are of the solid type. These four cases are presented.

The first involved the external condyle of the femur in a male aged 35. Trouble present two months. Condyle curetted and cauterized. Sections of the material showed compact hyaline connective tissue, apparently growing very slowly. Three months later, on account of suppurative arthritis and osteomyelitis, the leg was amputated. The specimen showed fleshy tumor tissue protruding from the external condyle, grossly looking like sarcoma. Sections of this showed an actively growing connective tissue with frequent mitoses.

The second case, a woman aged 53, had pain in the knee for several years following injury. Leg amputated ten years ago with diagnosis of osteosarcoma. A tumor about 7 cm. in length and 5 cm. in breadth involved the center of the tibia. The tumor was grayish white in color and of firm consistence. Sections

showed loose and compact connective tissue with rather frequent mitotic figures. In addition there was perforation of the cortex and appearance of fibrous tissue beneath the periosteum.

The third case, a female aged 15, had pain for eighteen months following injury. The base of the femur was rarefied. Cavity curetted. Material from the cavity was loose connective tissue. Sections showed in addition active destruction of bone by osteoclasts. Curettage done two and a half years ago. At the present time the condition is progressing and there is also involvement of the ilium.

The fourth case, a woman aged 38, had pain in the foot for twenty years. X-ray showed involvement of the cuboid and possibly of the cuneiform. Material from this area showed loose connective tissue.

Sections of the tissue from Cases 1 and 2, particularly, could readily be diagnosed sarcoma. Such cases as these have been amputated and reported as cures of osteogenic sarcoma.

(Discussion by Dr. James Ewing, New York.) I would like to ask if any of these cases would get well if treated by moderate repeated doses of X-ray.

(Dr. J. S. McCartney, Minneapolis, closing.) The girl of fifteen is the only one of our series who showed involvement of more than one bone (femur and ilium). She has been getting X-ray treatment ever since the operation in July 1923 and the disease is extending. The head of the femur is expanding and the areas in the ilium are gradually getting larger.

On the Pathology of Catarrhal Jaundice. Paul Klemperer and (by invitation) John A. Killian, New York.

Abstract. Clinical observations and various laboratory tests for disturbed liver function indicate that the functional capacity of the liver is severely altered in cases of so-called catarrhal jaundice. These facts are suggestive of anatomic changes in the liver parenchyma rather than inflammatory changes either in the large or small bile ducts. Owing to the benign nature of the disease anatomic data are extremely scarce. Only necropsy findings in the four cases reported by Eppinger can be accepted.

We have had the opportunity to observe a case of so-called "catarrhal jaundice" where an exploratory laparotomy was performed. The exploration of the bile ducts, gall bladder and pancreas gave no explanation for the intense jaundice. The liver was large but did not show biliary stasis. A small piece was excised for diagnosis. The outstanding microscopic findings were diffuse, severe parenchymatous degeneration with necrosis, primarily localized within the center of the acini. There was no cholangitis. The intracellular bile capillaries, however, showed ruptures due to the liver cell necrosis.

These morphologic findings corroborate the modern clinical conception that the so-called catarrhal jaundice is due to an injury to the liver cells.

(Discussion by Dr. M. J. Stewart, Leeds, Eng.) During the war, we saw in Leeds many cases of trinitrotoluene poisoning. We did eight or ten postmortems and in every one there was an acute or subacute atrophy of the liver. Some five years later I traced all the patients who had been in the Leeds Infirmary suffering from trinitrotoluene poisoning with jaundice, and who made recovery and all were in excellent health. In a case of salvarsan (Galyl) poisoning on which I made a postmortem I came to similar conclusions to those of Dr. Klemperer and Dr. Killian. A girl came to the venereal disease clinic with

syphilis and was treated with a course of five injections. At the end of the course she had a slight attack of jaundice which lasted three or four days. She came back to the hospital two months later to have venereal warts removed, and died twelve hours after operation. At postmortem she had typical subacute atrophy of the liver with scattered red areas of total destruction, while the rest of the organ which had been in the yellow stage was intensely fatty. That (major) portion of liver had survived the toxic onslaught and she was recovering but the undestroyed liver had been getting fatty and was unable to withstand the anesthetic. In the ordinary way this case would have been diagnosed as catarrhal jaundice. It was really one of acute atrophy of the liver with limited destruction of parenchyma in which recovery would almost certainly have occurred but for the using of a general anesthetic.

(Discussion by Dr. E. B. Krumbhaar, Philadelphia.) I would like to ask Dr. Killian the nature and extent of the van den Bergh reaction, if it was done, and also the condition of the Kupffer cells which, from the slides, were not involved.

(Dr. J. A. Killian, New York, closing.) In regard to the question just asked, that will be presented in the next paper.

CONTRIBUTIONS TO LIVER PATHOLOGY IN ACUTE YELLOW ATROPHY AND FATAL PHOSPHOROUS POISONING. John A. Killian (by invitation) and Paul Klemperer, New York.

Abstract. In both cases presented, the diagnosis was based upon postmortem findings, and in the case of phosphorus poisoning, phosphorus was demonstrated in the liver. In the three specimens of blood obtained from the acute yellow atrophy during the twenty-four hours before death in the hospital, there was noted a progressive rise in the nonprotein nitrogen of the blood. The amino and rest nitrogen of both the blood and urine was increased, but the urea nitrogen of the urine formed but 60 per cent of the total nitrogen. In the first specimen of blood, the urea nitrogen was within normal limits, but formed only 30 per cent of the nonprotein nitrogen. In the later specimens the urea nitrogen was increased above normal but formed but a small fraction of the nonprotein nitrogen. The ammonia nitrogen of urine was increased in proportion to the total nitrogen and this was associated with a mild acidosis. At the height of the toxemia there was observed a hypoglycemia. The infusion of 100 gm. of glucose produced a marked hyperglycemia persisting for more than two hours and accompanied by a glycosuria. In all specimens of blood the fibrin was low, but in the specimen obtained before glucose infusion no fibrin could be obtained from blood plasma. The patient had a marked icterus with a high icterus index and strongly positive van den Bergh reactions.

A child of 5 years was admitted about thirty hours after onset of sickness and about two hours before death from phosphorus poisoning. The blood and urine obtained upon admission show a low urea nitrogen in relation to total nonprotein nitrogen, high amino nitrogen, and (in the urine) ammonia nitrogen. There was a marked hypoglycemia and at this time the patient was in convulsions. The icterus index was increased to about eight times the normal, with a very strongly positive indirect, and a positive direct van den Bergh reaction.

(No discussion.)

THE PATHOGENESIS OF CIRRHOSIS OF THE LIVER. M. A. Goldzieher (by invitation), Budapest.

Abstract. A series of cases of liver cirrhosis has been studied. It has been observed that there are constant anatomic findings in some of the endocrine glands. The thyroid and the gonads are, as a rule, decreased in size. There is an excess of stroma in the thyroid and changes in the glandular parenchyma similar to atrophy. In the testicles beside the atrophy of the seminal tubules and hyalinization of their tunica propria, there is a hyperplasia of Leydig's cells present. The latter findings are identical with those in congenital underdevelopment. In the ovaries the changes found are comparable to those in the testicles.

The pineal of the cirrhotics is constantly larger in size and does not show those signs of involution which are regularly found in individuals of mature age.

There were less constant anatomic findings in other endocrine glands such as the hypophysis, adrenals and pancreas.

The interpretation of these findings points to a certain interrelation between endocrine function and regeneration. Liver cirrhosis is supposed to occur when the regenerative power of the liver is unable to cope with the repeated injuries. The important rôle of thyroid and gonad function in favoring regeneration generally has been proved experimentally. The pineal, as demonstrated by pathologic evidence, seems to have an inhibitory effect upon the gonads up to the age of adolescence. Destruction of the pineal in childhood leads to a development of secondary sexual characters and particularly to growth of hair on the body. Individuals with a persistent or hyperplastic pineal, such as is found in liver cirrhosis, are conspicuous for their hairlessness.

The theory has been brought forth that liver cirrhosis occurs only in such individuals whose constitution is anomalous. An underdevelopment of the thyroid and the gonads and persistency of the pineal inhibit the course of normal regeneration. Such anomalous individuals develop cirrhosis of the liver after chronic alcohol or other intoxications, while the livers of normal persons are capable of substituting the lost parenchyma through regeneration.

(Discussion by Dr. M. J. Stewart, Leeds, Eng.) There is a possibility which I think might be borne in mind; namely, that there are alcoholic drinks and alcoholic drinks. There is a striking difference between Scotland and England with regard to the incidence of cirrhosis. In eight years study in Scotland one saw very little cirrhosis of the liver. In England, in sixteen years one saw a great deal of it. A possible conclusion to be drawn from this is that the racial difference in incidence is due to racial difference in drinking habits. In England the stronger liquors drunk by the hospital class are gin and insufficiently matured whiskey. In Scotland, before the war, spirit drinkers, even among the poorer classes, drank only whiskey of good quality.

(Dr. M. A. Goldzieher, Budapest, closing.) This remark about the whiskey habit being prevalent in Scotland and the comparative rarity of liver cirrhosis seems to indicate that the Scotch have small pineals and good gonads and thyroids.

DECIDUAL CELLS IN BLOOD VESSELS. F. B. Mallory, Boston.

Abstract. In four cases of rupture of the uterus at a cornu as the result of interstitial pregnancy many of the blood vessels in the smooth muscle tissue were found to contain masses of decidual cells. This occurrence of decidual cells in

blood vessels during uterine and particularly during tubal pregnancy was observed as early as 1895 and several papers were written on the subject in the following ten years.

Study of decidual cells shows that they are formed in two ways, by transformation of proliferated fibroblasts, especially in the upper layer of the mucosa, and of existing fibroblasts, especially in the myometrium. The cell processes of the old fibroblasts retract and the fibroglia fibrils become wavy and corkscrew-like and in time disappear. The nuclei of these decidual cells often become multiple as a result of direct division forming giant cells which in the past have often been interpreted as syncytial cells that have invaded the uterus.

Evidently absorption of a chemical substance (a hormone) secreted by the chorionic epithelium stimulates the adjoining fibroblasts to proliferate and to change to decidual cells. Diffusion of this hormone from the uterus out through the oviducts may cause decidual formation in the mucosa of the tube and occasionally on the outside of the uterus.

Absorption of it around and within the blood vessels especially beneath the lining endothelium, may cause proliferation of the fibroblasts and transformation of them into decidual cells. In this way masses of them may be produced within blood vessels. Those in the veins may be carried away in the blood stream, those in the arteries are packed in the terminal branches of the vessels and occlude them.

In one case of chorionepithelioma of the uterus the tumor had invaded arteries and veins, but the numerous mitoses and the differentiation of Langhans' cells into syncytial cells rendered easy the recognition of the kind of cells in the blood vessels. In the second case the decision was more difficult. The tumor growth was slower and several arteries were packed full of decidual cells. In the myometrium were many large cells which might be either tumor cells or decidual cells, probably the former.

It is important to recognize the normal occurrence of decidual cells in the blood vessels of the oviducts and uterus during pregnancy, otherwise they may be mistaken for the tumor cells of chorionepithelioma.

(Discussion by Dr. Alfred Plaut, New York.) I wonder whether I was correct in understanding Dr. Mallory that in the so-called chorionic invasion there is no true chorionic invasion in uterine vessels but that these cells have to be considered decidual cells. Does this apply generally to all cases or to certain cases? Second, I should like to know whether there are substances which are responsible for the decidual change in tissue other than the endometrium, or whether absorption by lymph stream or the blood stream has to be considered. The decidual change in the cul-de-sac is found in a rather high percentage of all cases of pregnancy. I wonder if we always have to consider the way through the tube for this substance. Concerning the decidual change in connective tissue of the tube — a few years ago I saw a case like this one and I published it. I would not do it to-day because I have seen since that it is not so infrequent as I thought. It was more a direct change of the connective tissue cells of tubal folds than a multiplication of cells. It seemed that there must be some action of the decidual cells upon the covering epithelium because in looking through slides I was guided after a short experience by flattening of the epithelium and in the neighboring tissue found the decidual change. The swelling produced by decidual change was not sufficient to explain the flattening of the epithelium. Furthermore, edema which may give a picture simulating decidual change did not lead to this flattening of the epithelium.

(Discussion by Dr. V. C. Jacobson, Albany.) Occasionally in pregnant women one sees foci of decidual change in the tubal mucosa and in the ovary. Cases have been described in which there were multiple foci of decidual cells beneath the mesothelium of the pelvic viscera, including intestines, and without endometrial epithelium. The explanation of this phenomenon would seem to be that some hormone of pregnancy had acted on fibroblasts where it had come into contact with them, converting them into decidual cells in the same manner as Dr. Mallory postulates for his findings in and about uterine blood vessels. And another point of importance from Dr. Mallory's observations is that his identification of the cells found in the uterine vessels in some cases of chorionic epithelioma as decidual cells and not tumor cells, probably explains the good operative results in many cases, the cells which metastasized being benign decidual cells and not tumor elements.

(Discussion by Dr. M. A. Goldzieher, Budapest.) Dr. Mallory's paper is of great importance in those cases which have been described as chorionepithelioma in males. I believe that some of these tumors may be derived from teratomas and may really be classed with chorionepitheliomas. The majority of these, however, should be classed with giant cellular angiosarcomas and they are certainly altogether different from chorionepithelioma. The development of such decidual-like cells from endothelial cells is much more plausible and I think Dr. Mallory's work supports this point. I think that the formation of decidual cells is not always dependent upon the influence of hormones because in the cases of male chorionepitheliomas, there is no evidence of any hormone activity.

(Dr. F. B. Mallory, Boston, closing.) The observation of something which caused decidual cells to appear in the lymph nodes near the uterus has been made in the past and is well known. Another explanation of how decidual cells are formed beneath the mesothelium is stretching of the uterus so as to allow the direct escape of something which causes the decidual cells to form.

Myocardial Changes in Cardiac Defects. Maude E. Abbott, Montreal.

Abstract. Very little has been done to determine the histologic appearances of the myocardium in cardiac anomalies, although there is quite a rich literature on the inflammatory and degenerative changes that may occur in the myocardium of infants as a result of congenital cardiac syphilis. The most important studies are by Letulle (Presse Medicale) who demonstrated a chronic pericarditis in a number of cases examined and von Zalka (Frankfurter Zeitschr., 1924). The latter author examined fourteen cases of cardiac anomalies, four of which were from his own necropsy service, and of which eight were to be classed from the gross appearances as purely developmental in origin (cardiac septal defects) while six showed a stenosis or atresia of one or other of the semilunar orifices, suggesting an inflammatory origin. No changes were noted by him in the first group of so-called developmental cases, but in the other six cases of congenital valvular lesions he found extensive myocardial changes, and inflammatory processes in four and recent in two.

In several cases which have come recently within the personal experience of the writer of pulmonary or aortic atresia the myocardium of the ventricle giving off the occluded vessel showed macroscopically large yellowish gray areas unmistakably evidencing disease and which microscopically showed degenerative and inflammatory lesions resembling those described by other writers. The corresponding character of these lesions and their relationship to the proliferated and myxomatous endocardium (which presents a curious resemblance

to the embryonic endocardial cushions) seemed to us of sufficient interest to warrant the presentation of two of these cases here. The appearances in these cases appear to us to suggest that primary myocardial disease, probably of syphilitic origin has caused arrest or improper involution and irregular fusion of the primitive bulbar cushions and a failure of the muscular septal bands of the ventricle to invade these and that this may be in some cases the cause of conus stenosis.

(No discussion.)

The Pathogenesis of Old Valvular Defects. B. J. Clawson and E. T. Bell, Minneapolis.

Abstract. In addition to the vegetations in acute endocarditis there is a diffuse inflammation, always in the free edge and often involving the greater part of the leaflet. This circumstance explains the uniform thickening so commonly seen in old defective valves.

Rheumatic vegetations are composed chiefly of fibroblasts, and in the process of healing they readily become converted into fibrous tissue. There is no ulceration and no organization. Fifty-five of seventy-three old defective valves are considered the result of rheumatic endocarditis, and, in twenty-seven of these, incompletely healed rheumatic lesions were recognizable.

Bacterial endocarditis is a more intense inflammation than the rheumatic. Proliferation predominates but exudation is often prominent. Large thrombi are formed on the raw surfaces and there is often ulceration. Healing consists in the conversion of the leaflet into scar tissue. Such portions of the thrombi as do not become detached persist indefinitely without becoming organized, although they may become calcified. Complete healing rarely occurs. Three of seventy-three old defective valves were interpreted as the result of bacterial endocarditis.

Transitions between rheumatic and bacterial vegetations are frequently seen. Rheumatic vegetations were found in association with bacterial in three-fourths of the cases of subacute bacterial endocarditis.

Fifteen of seventy-three old defective valves belong to the aortic calcified nodular group. The etiology of this type is unknown. There is no satisfactory evidence that it is of inflammatory origin, and it seems unrelated to atheroma. Aortic stenosis in the absence of disease of any other valve is usually of this form.

Stenosis is more frequent than insufficiency in old defective valves.

The only old pulmonary valve defects seen were of the congenital type (3 cases of pulmonary stenosis).

An acute rheumatic endocarditis may terminate in several different ways: (*a*) death during the acute stage from toxemia; (*b*) partial or complete healing followed after a variable interval by the reappearance of fresh rheumatic vegetations (recurrent rheumatic endocarditis); (*c*) partial or complete healing followed by the formation of bacterial vegetations on the valves — a more active inflammation (subacute bacterial endocarditis); (*d*) slow incomplete healing giving rise to deformed leaflets on which rheumatic inflammation is still recognizable; (*e*) complete healing resulting in thickened, stiffened valves with smooth surfaces.

As to pathogenesis, seventy-six hearts with old valvular defects are interpreted as follows: fifty-five from rheumatic endocarditis, three from bacterial endocarditis, fifteen (all aortic stenosis of the calcified nodular type) of undetermined origin, and three (pulmonary stenosis) congenital.

(Discussion by Dr. A. M. Pappenheimer, New York.) I wish to ask Dr. Bell whether he has frequently found transitions between the rheumatic and the bacterial types of endocarditis. We have recently observed a case in which the two types of valvular disease coexisted. On one surface of the mitral leaflets were frank bacterial vegetations, with colonies of micrococci and a profuse cellular reaction; on the opposite surface were small vegetations of the rheumatic type without demonstrable bacteria. The myocardium likewise showed two distinct types of lesion. In addition to focal necrosis and cellular infiltrations such as are usually found in bacterial endocarditis, there were characteristic Aschoff bodies. Thus, although the two types of infection may coëxist, and each in an active form, it has always seemed to us fairly simple to differentiate them histologically.

(Dr. E. T. Bell, Minneapolis, closing.) Sometimes one sees valves in which the rheumatic vegetation is on one leaflet and the bacterial on the other. As I mentioned, if you study all subacute cases and look over the leaflets very carefully, you will find rheumatic vegetations along with the bacterial in about three-fourths, and usually on the same leaflet. If one wants to say that infectious subacute endocarditis is a different infection from rheumatic, one must assume a rheumatic infection complicating three-fourths of the cases of subacute endocarditis.

Specific Lesions of Peripheral Blood Vessels in Rheumatism. W. C. VonGlahn and A. M. Pappenheimer, New York.

Abstract. In ten of a series of forty-seven consecutive cases of rheumatic carditis, vascular lesions of a specific type were observed in the following situations: lungs, aortic valve, kidneys, perirenal and periadrenal adipose tissue, appendix epiploica of sigmoid colon, testis, pancreas and polyp of cecum. In the lungs, practically every small branch of the pulmonary artery was affected; in other situations only isolated vessels.

The lesions are characterized by exudation of fibrin into and about the vessel, by necrosis of the cellular elements of the vessel wall, and by a very characteristic cellular reaction in the surrounding tissue. These acute changes are followed by organization, often with the formation of new blood channels within the thickened intima, and occasionally in the media. Absence of thrombosis was a characteristic feature. The lesions differ from the commonly described forms of arteritis, and are regarded as of rheumatic origin.

(No discussion.)

Multiple Necroses of Spleen (Feitis) in Pernicious Anemia. O. T. Schultz and (by invitation) Norbert Enzer, Chicago.

Abstract. In 1921 Feitis described a condition which he termed Fleckmilz. The spleen in two cases of cardio-renal vascular disease was studded with grayish white nodules, varying in size from such as were just barely visible to others larger than a linseed. Microscopic examination proved these areas to be necroses which involved the Malpighian bodies. Since then a similar condition has been reported by Geipel and by Mathias, each of whom had seen a single instance in eclampsia, by Wilton in a case of pneumonia, and by Meuret who saw the condition in two cases of cardio-renal vascular disease.

A case of pernicious anemia, in a woman aged 59 years, is presented, in which the spleen contained multiple necrosis of the type described by Feitis.

The spleen was small, measuring 9 by 5 by 3 cm. On the cut surface were numerous grayish areas 1 to 2 mm. in diameter, which were believed to be hypertrophied Malpighian bodies. Microscopically these were areas of coagulation necrosis, each involving a Malpighian body. In the sinusoids were large phagocytic cells which contained fragments of nuclei and erythrocytes. These cells were especially numerous in the sinusoids immediately about the Malpighian bodies.

In the previously reported cases the necrosis of the Malpighian bodies began at the center and was due to occlusion of the arteries of the corpuscles. In the case here reported the necrosis began at the periphery of the body and was due to filling of the sinusoids of the peripheral zone of the Malpighian bodies by phagocytic reticulo-endothelial cells.

(Discussion by Dr. Alfred Plaut, New York City.) Did these large cells contain fat or lipoid?

(Dr. O. T. Schultz, Chicago, closing.) The cells in question did not contain lipoid. Although probably of the same origin, they were not identical with the large, lipoid-containing reticulo-endothelial cells which may be so numerous in the condition which Bloom has termed lipoid-histiocytosis. Their situation also differed in the two conditions. In lipoid-histiocytosis most of the cells are free in the pulp between the sinuses, whereas in the case herewith reported they were present within the sinuses. In the latter case they were filled with particulate matter derived from erythrocytes and leucocytes.

THE VASCULAR MECHANISM OF THE SPLEEN. W. L. Robinson, Toronto.

Abstract. In order to demonstrate the histologic structure of the spleen and the nature of the blood flow through it, sheep and dog spleens were injected via the arteries and veins with a variety of solutions. In all cases the detail structures were shown up quite readily by injecting the pulp to capacity through the vein with fixing solutions such as Zenker's and 10 per cent formalin. To demonstrate the nature of the arterial circulation a warm 25 per cent gelatine solution with or without carmine pigment was found the most satisfactory. Varying amounts of these solutions were injected with the object of grading the injections to determine the course of the fluid mass through the vascular and pulp systems. The gelatine *in situ* was satisfactorily stained with iron hematoxylin, providing that the differentiation was not carried too far. In the cases where carmine pigment was added to the gelatine, picric acid was used as a counterstain instead of picro-acid fuchsin mixture.

The arteries were found to occupy the central portions of the splenic lobules as outlined by Mall. Branches from these penetrated the lymphoid corpuscles of Malpighii and divided into a number of very fine branches of capillary size and structure supported by the pulp cells. In all cases they were found to terminate abruptly in a pear-shaped end-organ, the ellipsoid of Schweigger-Seidel. This was found to consist of a group of cells, endothelial in type and function, covering the end of the fine capillary branches and supported directly by the pulp cells. The portion of the capillary surrounded by these cells was quite permeable to the gelatine solution, slightly less to the carmine pigment. The surrounding ellipsoid cells, while appearing as compact masses, were found on injecting the gelatine solutions, to have intercellular spaces allowing for the flow of the injection mass from the ellipsoid capillary to the pulp and venous sinuses. The ellipsoids were found to have a very intimate relationship to the

pulp spaces and most of our injected material was found to flow directly into the pulp spaces. On the other hand the ellipsoids were found, in a number of cases, to be lying in direct contact with venous sinuses. Such close contact allowed a direct flow from ellipsoid through the intercellular spaces of the venous sinuses. These same venous sinuses were also found to have free communication with the pulp spaces by their slit-like stomas.

We are of the opinion that the vascular mechanism of the spleen is of the nature of an open circulation — the bulk of the blood flowing from ellipsoid through the pulp spaces to the vein. On the other hand much of the flow is more or less direct from the ellipsoid into the venous sinuses through their intercellular spaces.

(Discussion by Dr. H. T. Karsner, Cleveland.) The necessity for reproducing many of our pictures in black and white means very often that the beauty of the demonstration is obscured. I have had the privilege of seeing some of Dr. Robinson's preparations in his laboratory in Toronto and I wish to say that as convincing as this demonstration is in black and white, a view of the original preparations, or series of preparations, is much more so.

(Discussion by Dr. E. B. Krumbhaar, Philadelphia.) There have been numerous physiologic examples recently described where by means of shuntings, alternating activity or other expedients, the circulatory needs of an organ can be kept at a minimum for average purposes and yet quickly adapted to much greater needs. I need only instance Krogh's capillaries and Richards' work on the glomeruli of the frog's kidney. Dr. Barcroft's recent work on the spleen would indicate that such mechanism was peculiarly needed in the spleen. You may recall that he showed that there was very extensive reservoir power of the spleen for blood, whereby in response to demands, such as exercise, carbon monoxide poisoning, etc., very considerable amounts of blood could be brought out into the circulation. In a cat, for instance, with a very "muscular" spleen the number of corpuscles sent into the general circulation amounted to one-third of the total volume of the blood cells of the body. In some cases, as in carbon monoxide poisoning, where the hemoglobin carrying power was affected, the presence or absence of the spleen was shown to make the difference between life and death. It thus becomes a practical matter of some importance. It is extremely desirable, of course, if such is the case, to have as many ways of getting the blood circulating through the spleen as possible, and it is particularly pleasing to hear Dr. Robinson's demonstration showing that, as I understood him, blood can come through the "open" circulation of the pulp as well as by direct communication between the arteries and the small arterioles and the sinuses. Possibly Dr. Robinson is familiar with Braus' Anatomie der Menschen, recently published, in which he presents a diagram showing numerous ways in which the blood may get from the arterial to the venous system, by (1) direct outpouring from the arteries into the pulp, or (2) through the fenestrated ends of the arteries into the pulp, or (3) by direct communication of artery with the venous sinus. In the same way blood is taken up by the sinuses either through trumpet-shaped openings or through the sinus fenestrations. Such an arrangement, with its various controllable mechanisms for holding back or hastening the blood through the spleen, would fit in well with the functional observations previously referred to. I would like to ask what connection these ellipsoids of the arterial capillaries have with the Husenarterien and the penicillia of Ruysch.

(Discussion by Dr. E. M. Medlar, Madison.) This gives the structure of the histologic unit and is interesting. I wonder if the author has also studied the capillary distribution in the Malpighian corpuscles.

(Dr. W. L. Robinson, Toronto, closing.) At no point was I able to demonstrate a direct connection between the artery and vein. I know some believe that such occurs, but I think these can be explained by the presence of a vein running parallel to the ellipsoid and cut tangentially. At no point could I trace a thread of gelatin through to a vein. In the dog, I was of the opinion that the flow in all cases was through the ellipsoid. As far as the Malpighian bodies are concerned, I could demonstrate ellipsoids in many of them. Some claim that they are unable to inject the Malpighian bodies. In many cases I found that I could do it quite readily.

The Open Circulation of the Spleen Pulp. Ward J. MacNeal, New York.

Abstract. In the study of spleens obtained at surgical operation or at necropsy, the gross examination is completed promptly. After taking a culture specimen by capillary pipette, a small portion of the organ is removed at one end, for preparation of smears and for fixation in the collapsed state. A cannula is next introduced into an arterial branch near the other end of the spleen and this is perfused with Locke's solution with addition of 1 per cent sodium citrate and 0.25 per cent gelatin; followed by a little salt solution and then Helly's formalin-Zenker fixing solution, so as to fix the spleen in a moderately distended state. Near the other end one makes a similar injection into a tributary of the splenic vein. There are thus available for study (1) smears, (2) sections of undistended spleen, (3) sections of spleen distended by artificial perfusion, (4) sections of spleen distended by venous perfusion.

In attempting to ascertain the connections between arterial and venous capillaries the nucleated erythrocytes of birds have been injected through arterial branches into very fresh human spleens and have been introduced through a gastric branch of the splenic artery into the living spleen of anesthetized animals, with subsequent precisely timed fixation.

The sections of distended human spleen have so far not revealed any recognizable direct continuity between arterial and venous capillaries. It is easy to recognize the terminations of arterial capillaries in the spleen pulp, especially in the marginal zone of the Malpighian corpuscles. The terminations are usually distended and the lumen communicates with the pulp spaces between protoplasmic strands of branched reticulo-endothelial cells. In the pulp cords between the venous sinuses, arterial capillaries are also found. They are longer than the others. They also terminate with an ampula in the pulp, but with very little substance intervening between the ampula and the adjacent venous sinuses, so that an injection mass might readily give the impression of direct continuity. From the pulp there are many minute openings into the venous sinuses, especially the smaller ones adjacent to the marginal zone of the Malpighian corpuscle.

The experimental injection of bird's corpuscles with subsequent prompt fixation has conclusively demonstrated that these corpuscles pass readily from the arteries into the spleen pulp, especially in the marginal zone and from this through the wall of the venous sinuses to gain the venous lumen.

The observations are in close agreement with the conception of Weidenreich and are distinctly opposed to the conclusions of Helly in regard to the circula-

tion of the spleen pulp. The blood passes here in spaces without defined vascular walls. The circulation is in this sense an open one.

(Discussion by Dr. L. U. Gardner, Saranac Lake.) May I ask either of the former speakers whether there is any information available as to the comparative structure of the spleen of the guinea-pig and the rabbit?

(Dr. W. J. MacNeal, New York, closing.) I have no information on guinea-pigs at all. We have undertaken to study a few guinea-pig spleens but none of the material is ready. So far I can speak only of human and rabbit spleens.

Observation of Formation of Giant Cells in Turtle Blood Cultures. Mortimer Cohen (by invitation), Pittsburgh.

Abstract. Observation was carried out on hanging drop cultures of turtle blood. Some of the cultures were made in accordance with Lewis' method, others were vitally stained with neutral red and Janus green and in a few instances, blood from turtles which had been injected with India ink was used. Individual cells and groups of cells were studied for as long as twenty-six days. During the periods of greatest activity the microscopic fields were not changed and almost continuous observation was carried out. Quiescent mononuclear cells with undifferentiated protoplasm were observed to develop Brownian movement, rod-shaped mitochondria, to send out pseudopodia and to take on the characters of wandering cells. Multinucleated giant cells were observed to form by fusion of large mononuclear wandering cells. Nuclear division was not seen. No attempt was made to deterimne the nature of the cells which fused to form giant cells. In later cultures multinucleated giant cells were seen to give off multinucleated masses which continued to live for some days as individual multinucleated giant cells.

(No discussion.)

Giant Cells and their Relation to Caseation in Tuberculosis. E. M. Medlar, Madison.

Abstract. From this study the "giant cell" in tuberculosis is found to be similarly produced in guinea-pig, fowl and man. The material which has been considered as its cytoplasm is in large part composed of necrotic tissue or of caseous exudate. In this dead material particles of reticulum can commonly be demonstrated. The "giant cell" is formed by mononuclear leucocytes wandering into and surrounding the area of necrotic exudate. The compactness of the dead substance apparently determines the arrangement of these cells and the consequent arrangement of the nuclei. Evidence of these cells wandering into the "giant cell" can be demonstrated. Particles of coal pigment in "giant cells" have been found. This demonstrates that mononuclear leucocytes which have previously phagocytized coal pigment at times participate in "giant cell" formation.

Lymphocytes have also been found within "giant cells."

It is apparent that "giant cell" formation follows rather than precedes areas of caseation or of necrosis. The mononuclear leucocytes and to a less extent the lymphocytes invade or surround these "foreign bodies" for the purpose of removing them by phagocytosis or by digestion from the tissue and thus promote complete repair.

(No discussion.)

A Study of the Process of Caseation in Tuberculosis. E. M. Medlar, Madison.

Abstract. This study shows the similarity of the process of caseation in the guinea-pig experimentally infected and in fowl and human hosts naturally infected. The primary reaction in all of these hosts to the tubercle bacillus is the formation of the mononuclear or "epithelioid" tubercle without giant cell formation, caseation or polymorphonuclear leucocytic infiltration. Lymphocytes play but little part in this phase.

If the resistance of the host is high and the dosage or the virulence of the tubercle bacillus low, hyperplastic tuberculosis results. This may go on to healing and fibrosis without necrosis, caseation or giant cell production intervening. In this reastion the polymorphonuclear leucocyte plays no part.

Following either an injury to the mononuclear leucocytes, or the production of a substance or substances by the growth or by the disintegration of the tubercle bacillus, the polymorphonuclear leucocyte is attracted. These cells accumulate in relatively small or in large numbers. For some reason they are early injured and die without the production of a typical abscess. These dead cells form the bulk of the caseous material.

At this stage, if sufficient autolysis has occurred and if opportunity for extrusion of the exudate is afforded, cavitation or ulceration ensues. If this does not occur, mononuclear leucocytes and lymphocytes wander into the caseous mass and attempt digestion and removal of the material. If the area be small, scar tissue may be the end result. If the area be large, calcification may be the end result. If the tubercle bacilli are not destroyed the process outlined above will occur in adjacent tissue with a consequent tissue destruction and spread of infection.

After an area of caseation is produced the polymorphonuclear leucocyte does not appear to be further attracted.

(No discussion.)

Healing in Intestinal Tuberculosis. Leroy U. Gardner, Saranac Lake.

Abstract. The following observations are based upon a study of necropsy material from sixty-one cases dying usually from far advanced phythisis. Of this number, 46 or 75.4 per cent showed some evidence of intestinal involvement; of the 46 cases, 18 or 39.1 per cent showed gross or microscopic evidence of healing. The degree of healing, with a few exceptions, corresponds to the duration of treatment with the quartz mercury vapor lamp. Eleven cases receiving no heliotherapy were also studied. In two of these there was evidence of healing.

The process of healing consists in the arrest of the specific tuberculous foci or their destruction in the course of ulceration. This is followed by the development of an inflammatory granulation tissue in the floor of the ulcer which resolves without excessive scar formation. The denuded surface is covered by epithelium which regenerates from the border of the affected area. Occasionally where the destruction of tissue has been excessive, teat-like mammillations are found, sometimes singly and sometimes in clusters which persist apparently for an indefinite period to mark the site of former reaction. Such healing is accomplished with the development of stenosis from cicatricial contracture or of adenomatous growths. The only gross deformities produced are variable degrees of thinning and dilatation of the intestinal wall from destruction of the muscular coats and the previously mentioned mammillations.

(Discussion by Dr. M. J. Stewart, Leeds, Eng.) I would like to ask what happens to the muscularis mucosae in healed ulcers. To what extent does deep penetration by glands occur?

(Dr. L. U. Gardner, Saranac Lake, closing.) In reference to the point of destruction of muscularis, there is apparently no attempt to regenerate smooth muscle cells. A break in the muscularis is filled in by granulation tissue which ultimately remains. In regard to deep penetration by glands — yes, penetration goes down to the outer third of the submucosa but I have not observed it to go any further.

The Histopathology of the Subcutaneous Lesions in Tularemia. Howard H. Permar and Grover C. Weil, Pittsburgh.

Abstract. The materials forming the basis of this paper were obtained by biopsy from a woman of 45 years admitted to the Mercy Hospital, Pittsburgh, Pa., in the tenth week of the disease. The history and clinical course were typical and the diagnosis was confirmed by agglutination tests. The available lesions consisted of subcutaneous nodules located along the lymphatics of the arm.

The tissue changes are typical of a granuloma and may be summarized in sequence as follows. The earliest reaction consists of massing of endothelial leucocytes with giant cell formation. The local capillaries show endothelial hyperplasia with narrowing and obliteration of their lumina. As a result of the narrowing and occlusion of capillaries, areas of bland necrosis are formed, involving the endothelial and giant cell collections. Bacterial toxins may be an added factor here. Many polymorphonuclear leucocytes migrate to the necrotic focus which goes on to liquefaction. The adjoining fixed tissues give rise to a cellular granulation tissue in which fibroblasts predominate while capillaries are rather few and narrow. Endothelial hyperplasia also occurs in the new formed capillaries. Secondary lesions appear in the inflammatory wall and these go through the same stages, leading to the formation of necrotic foci which tend to fuse with the primary one. In the meantime, the entire lesion is gradually surrounded by a zone of lymphocytes and plasma cells. The healing process is one of slow organization.

We have had an opportunity to study sections of inflamed axillary lymph nodes from two additional cases of tularemia in Pittsburgh. Both showed tissue reactions essentially the same as those we have described. The literature contains no adequate presentation of human tularemia, though Councilman and Strong in detailing the acute lesions in animals, noted fundamental reactions identical with those we have described in the human. The granulomatous character is seemingly not evident in animals since in them tularemia is an acutely fatal infection.

(Discussion by Dr. G. C. Weil, Pittsburgh.) It seems to be the prevailing impression that surgical intervention in the treatment of tularemia is rather a dangerous procedure. However, several of the lesions in this case were attended by rapid healing following incision and drainage as compared with the other lesions in which no drainage was established. We were rather impressed also by healing by primary union of the lesions which were totally resected and without further recurrence. Marked improvement of the condition of the patient and the healing of the wounds was noted upon administration of potassium iodide and Fowler's solution.

(Discussion by Dr. W. L. Holman, Toronto.) I had an opportunity of seeing in San Francisco in the Public Health Laboratory a good many animals with

tularemia and with plague. The technician there, when he looked at the spleen of an infected animal, said the tularemia could be told very rapidly by just feeling the spleen. It felt granular, as if little grains of sand were scattered through the spleen. In the case of plague the infected areas are soft and abscessed. It is therefore very interesting that this lesion is a granuloma which would confirm this observation of the technician who had been working with plague about eighteen years and had used this rather simple method of differentiating. It is also interesting that these cases in the eastern part of the continent practically all come from rabbits. I do not think there is a human case from the original ground squirrels, in which the infection was first found by Dr. McCoy. It is particularly interesting that we have not found human beings infected from the ground squirrel.

(Dr. H. H. Permar, Pittsburgh, closing.) I think I have nothing particular to add. The course of the disease clinically would lead one to expect a granulomatous reaction. Our patient was ill for two and a half months and has just left the hospital. Some of the English workers who became infected in the Lister Institute were ill for as long as a year and a half.

Supravital Staining of Cultures of Lymph Node and Liver Endothelium. Frank A. McJunkin, St. Louis.

Abstract. In an investigation of the origin of the mononuclear phagocytes of the tissues and blood the reaction to neutral red of lymph node and liver endothelia growing *in vitro* has been determined. One of the mononuclear phagocytes is the peroxydase reacting monocyte of Naegeli which is found in normal human blood. The term has incorrectly been applied to cells that do not give the peroxydase reaction. Sabin, Doan and Cunningham were the first to observe and describe a second variety of phagocytes which they encountered in examining rabbit peritoneal exudates. It is a non-peroxydase reacting cell. On supravital staining with neutral red it is characterized by an accumulation in the cytoplasm of dye granules in the form of a rosette. The author found the "rosette" cell to be present in great abundance in the juice expressed from lymph nodes. In lymph nodes stained supravitally, fixed in Zenker-formol and sectioned, the rosette cells were found to be the reticulo-endothelium. A third type of phagocyte which has little affinity for neutral red was demonstrated in rabbit peritoneal exudates by the author. To study further the reactions of the phagocytes to neutral red the lymph nodes and the liver of rabbits were grown in tissue cultures.

The groin lymph nodes of normal rabbits and of rabbits injected subcutaneously with India ink were grown in the plasma of the same rabbit. Besides the lymphocytes, there appeared in the cultures two larger cells, the fibroblasts and the reticulo-endothelial cells. When treated supravitally with neutral red the former showed only a few scattered neutral red granules in contrast with the latter which accumulated the dye in eccentric spherical masses, or rosettes. The reticulo-endothelial cells phagocytize the carbon which is situated in the cytoplasm outside the rosette. The fibroblasts contain no carbon or only a few small carbon particles.

The liver of rabbits injected intravenously with India ink gave outgrowths into the plasma clot which consisted of two types of cells, the fibroblasts and the reticulo-endothelium. When treated with dilute solutions of neutral red the fibroblasts were observed to react with the accumulation of a few scattered dye granules and the reticulo-endothelium reacted not at all or with the forma-

tion of neutral red granules scattered irregularly throughout the cytoplasm. In the older cultures the neutral red granules became quite abundant in the reticulo-endothelial cells but there was no tendency for them to be grouped as a mass or rosette. The reticulo-endothelial cells contained much carbon. In both lymph nodes and liver cultures the reticulo-endothelial cells were round or elongated and connected by cytoplasmic processes which differed from the multiple tapering branches of the fibroblasts. They have more the appearance of the processes connecting the reticulo-endothelial cells of the lymph node sinuses or the attenuated prolongation of the endothelial cells of granulation tissue. Repeated attempts to obtain growths of normal liver failed.

Conclusions. 1. By the method of supravital staining with neutral red there are two kinds of endothelium: the lymph vascular type that accumulates the dye in the form of an eccentric mass or rosette and the blood vascular type that does not react at all to the dye or reacts with the formation of a diffuse granulation.

2. The individual phagocytes that separate from these two varieties of endothelium appear to retain the characteristics of the parent tissues.

3. The two endothelial phagocytes are non-peroxydase-reacting cells and should not be confused with the monocyte of splenomyelogenous origin.

(No discussion.)

METHYLENE AZURE B IN STAINING SECTIONS OF HEMATOPOIETIC TISSUES. Ward J. MacNeal and (by invitation) Sadao Otoni, New York.

Abstract. When methylene azure B (trimethyl thionin) was first prepared in our laboratory it seemed to possess no special advantage as a stain over the methylene azure A mixed with methylene blue. The tests then made related especially to its use as a blood stain in alcoholic solution according to the technic of Leishman.

Since that time methods have been perfected for the preparation of trimethyl thionin in comparatively large amounts and we have had opportunity to try this substance in the staining of tissue sections, with highly satisfactory results in sections of spleen and bone marrow. In combination with phloxine or with eosin following fixation in Zenker's or Helly's fluid, it gives an extremely crisp differentiation of the nucleus and of various protoplasmic granules and the hemoglobin content of various cells is distinct. It has proven to be particularly serviceable for the differentiation of neutrophilic granules, for which purpose it is much superior to anything else we have tried.

The technic is as follows. The tissues are fixed, best by perfusion through the arteries, with Zenker's solution or with the formolized Zenker solution of Helly, followed by immersion in the fixing solution for 2 to 6 hours. Wash in running water 48 hours. Embed in paraffin and cut at 2 to 5 microns. Float the sections on water and fix to slide or coverglass with albumen fixative. Dry thoroughly overnight at 37 C to insure adhesion of the section. Carry the section through xylol and graded alcohols to water, then to very dilute Lugol's solution for 15 minutes; wash and place in decinormal sodium thiosulfate for 5 minutes and wash in water. This removes precipitated mercury from the tissue. Stain in 1 per cent phloxine or eosin ten to sixty minutes; wash and stain in 1:1000 azure B bromide with gentle agitation for one-half to five minutes until the section is blue. Wash in water and carry through graded alcohols to 95 per cent alcohol containing a little colophonium, where the section is allowed to bleach to the desired tint; then to absolute alcohol, xylol and balsam.

Somewhat better differentiation may usually be obtained by the technic described by Bensley for the granule staining in the islands of Langerhans. after staining with the azure B the excess of stain is removed carefully with filter paper without washing; dehydrate in acetone; then to toluol; then differentiate in absolute alcohol one part, oil of cloves three parts; then toluol and balsam.

(No discussion.)

Hyperglycemia as Result of Vital Staining with Certain Dyes. The Relation of the Microscopic Distribution of the Dye to the Blood Sugar Level. Isolde T. Zeckwer, Boston.

Abstract. In a study of the factors concerned in the production of hyperglycemia, the method of vital staining was employed. Three dyes were found to cause a marked fluctuation in the sugar content of the blood: methylene blue, neutral red and safranin. After intraperitoneal injections of these dyes, there is a rapid rise of the blood sugar up to 200 to 300 per cent over the orginal value attaining a maximum in one to three hours, and declining to normal in about eight hours. Such response was invariable in rabbits, guinea-pigs, cats and dogs.

The immediate symptoms after injection of these dyes are flushing of the vessels of the ears, increased force of the heart, heightened blood pressure and increased force of respiration without evident asphyxia. Repeated injections of neutral red into the same animal cause no difference in the immediate reaction and no permanent increase in the blood sugar level. After a time nutrition is interfered with, the animals losing weight although they continue to eat well.

The dyes, after injection, are rapidly absorbed into the blood stream and stain diffusely all the tissues. They are eliminated into the lumen of the stomach and small intestine, and differ in this respect from other dyes studied, which were not eliminated by this route and failed to affect the blood sugar level. Methylene blue on account of its reduction to the colorless form, is difficult to trace histologically in individual cells, but the microscopic distribution of neutral red can be clearly seen. Aside from its presence in the organs to which all foreign material is carried, it is characterized by appearing in granular precipitate form in cells forming proteolytic enzymes, namely acinar cells of the pancreas and epithelial cells of stomach and small intestine. This seems to be of significance in view of the fact that it is known that neutral red and safranin combine chemically *in vitro* with proteolytic enzymes, forming a colored precipitate which contains all the enzyme. Methylene blue also was found to give a precipitate with trypsin *in vitro*, while the dyes not affecting the blood sugar level gave no such precipitate.

Epstein, in his recent work tending to show that trypsin neutralizes insulin, found that safranin when injected directly into the pancreas failed to cause an increase in blood sugar while all other substances so injected caused hyperglycemia. He accounts for this finding by suggesting that safranin fixes the enzyme within the cells and thus prevents the escape of trypsin into the blood stream, which according to his hypothesis would neutralize insulin. In the present experiments in which the vital staining of the entire animal is produced, the blood sugar curve is quite similar to the curve of hyperglycemia following injections of trypsin into the unstained animal, and the rapidity of the rise and fall in blood sugar, and the attendent circulatory phenomena would indicate that the

effect is one of increased glycogenolysis by way of a nervous mechanism rather than by failure in utilization of glucose due to neutralization of insulin.

These dyes appear then to have two actions: (1) to combine chemically with intracellular proteolytic enzymes and (2) to cause hyperglycemia apparently by way of the autonomic nervous system. That these two actions of the dyes bear any relation to each other seems probable but remains to be proved.

(No discussion.)

Hyaline Degeneration of Arterioles and Capillaries: Experimental Production in Animals. Herbert U. Williams and (by invitation) P. T. McIlroy, Buffalo.

Abstract. Hyaline degeneration of the arteries of the lymph nodules of the spleen may be found in practically every human subject 35 to 40 years old. In cases of arteriosclerotic kidney, hyaline degeneration of the interlobular arteries is usually present, which makes this degeneration of great practical importance. Hyaline degeneration of the arteries of the spleen nodules is also frequent in children from the fifth year on, and is seen in connection with a great variety of infections and intoxications; small areas of necrosis in these nodules often appear in the same cases. The hyaline change may appear in arterioles of smallest size, practically capillaries, and the lumen may be greatly narrowed and even obliterated. Degenerative changes seem insufficient to account for the mass of new material that appears under the endothelium. Study of arteries in spleen nodules gives the impression that the new material may well be in large part some substance derived from blood plasma; the association of the condition with infections and intoxications, especially in children, suggests that an increase in the permeability of the intima may permit the passage of such a substance into the vessel wall. The experimental work of the writers has been done on cats. Hyaline masses, sometimes quite large but usually small, are frequent in the lymph nodules of the spleen of the normal cat, being present in five-sixths of the normal adult cats examined; they are absent in kittens. Often an arteriole or capillary may be seen near the center of the hyaline body; frequently a hyaline thickening of a capillary wall may be demonstrated. In attempting to produce similar changes in cats the writers have used chiefly arsenic trioxide and histamine. The former was selected in the hope that the arsenic, or products of its action on other tissues, might be shown to have injured the lining of the small vessels and thus to have increased their permeability. It was thought that histamine would lead to dilatation of capillaries and possibly injure the lining also, and thus promote exudation of some of the constituents of the plasma. Both agents were given hypodermically, sometimes only using one of the two in a certain animal, sometimes using the two in one animal at successive injections. In order to eliminate the possibility that hyaline masses might already be present, a bit of spleen was removed by operation and examined before beginning the injections. Very young cats were also used to some extent without preliminary operations, but they proved very refractory to the treatment. In general, in spite of many negative and doubtful results, it seems that either arsenic or histamine, but better arsenic followed by histamine, may produce hyaline masses associated with the walls of arterioles and capillaries in the spleen nodules; sometimes the picture seen in human spleens of hyaline degeneration of capillaries was reproduced. Frequently, the morphologic relations and appearance of the new material made it practically certain that

it originated as an exudate. Small areas of necrosis were sometimes observed and in such cases the picture was closely like that seen in such human infections as scarlet fever. No certain results were obtained in vessels of the spleen that could definitely be called "arteries."

(Discussion by Dr. W. J. MacNeal, New York.) I am interested in this. I can confirm the occurrence of changes of this kind in the small arterial vessels of the spleen in very young children. In one such case the child had a severe hemorrhagic purpura with very low count of blood platelets. Following the splenectomy there was definite clinical improvement and increase in the platelets of the circulating blood.

(Discussion by Dr. H. T. Karsner, Cleveland.) In some work with Hanzlik several years ago, we pointed out that if a sufficient time elapse following injections of histamine, platelets are clumped in large masses in the pulmonary capillaries. It is at least conceivable that some of the masses along the capillary walls and vascular spaces may be clumped platelets.

(Discussion by Dr. Alfred Plaut, New York.) I would like to know about the reaction of these masses. Was fibrin stain or Russell's hyalin stain used? I would call attention to a few slides of mine which are on exhibition outside and which show changes related to the ones described in Dr. Pappenheimer's paper this morning.

(Dr. H. U. Williams, Buffalo, closing.) We have not tried the fibrin stain on every one of these, but tried it on quite a lot. There was no suggestion of the morphology of fibrin in any of them. I doubt if Dr. Karsner's suggestion as to thrombi of platelets will help. We had thrombosis in mind, and we considered various other explanations and have given them all up. I will keep that in mind, Dr. Karsner, and am obliged for the suggestion.

EXPERIMENTAL EMBOLIC GLOMERULONEPHRITIS. B. J. Clawson, Minneapolis.

Abstract. Fourteen rabbits were injected in the left ventricular cavity from one to five times with finely ground agar which had been heavily seeded with streptococcus viridans. Sixteen rabbits were similarly injected with agglutinated streptococci. Glomerular injuries were produced similar to those found in human kidneys in cases of subacute bacterial endocarditis.

(Discussion by Dr. H. T. Karsner, Cleveland.) The importance of this contribution is great and the features I wish to suggest are to be regarded in the light of constructive criticism. Successful experiments are reported with agar infiltrated with streptococci and we have no information as to what happens if sterile agar be injected. The paucity of lesions in those animals into which streptococci clumped by agglutinating serum were injected, raises the question as to whether or not streptococci clumped by other methods, as for example, acid agglutination, might not produce more frequent lesions of the glomeruli. It is at least possible that the agglutinating serum may so injure the organisms as to inhibit their pathogenic capacity.

(Dr. B. J. Clawson, Minneapolis, closing.) I should have mentioned that controls are run with sterile agar alone and so far no lesions have been produced. I think that agglutination produced by any method would give as good results as we have here. What we need to have is the organism stopping within the glomeruli. If you can get the organism to stop in the glomeruli they will set up a proliferation rather than an exudation. Any type of agglutination would serve, I should think, but I have not tried it.

Histologic Changes in Exophthalmic Goiter Following the Administration of Iodine. Alfred S. Giordano, South Bend.

Abstract. This study was made from a group of sixty-eight thyroid glands removed from exophthalmic goiter patients, who had received iodine before operation. As a basis of comparison, the author used glands taken from patients who had died following a typical exophthalmic goiter crisis. The study reveals very striking involution changes from an active hypertrophy and hyperplasia to a colloid type of goiter. On reviewing the clinical histories of these patients and comparing the histologic picture of the respective glands it was found that the degree and extent of involution was closely paralleled by and corresponded to the clinical improvement of the symptoms. The changes are similar in character as those described following ligation of the thyroid vessels, but occur rather uniformly throughout the gland.

(No discussion.)

Articles Read by Title

A Satisfactory Method for the Staining of the Nerve Fibers of the Iris. M. Balado (by invitation), Rochester, Minn.

Abstract. 1. Fixation: The eyeball is fixed in 10 per cent formalin for twenty-four hours. Transverse section of the eyeball in two parts, anterior and posterior. Detachment of the choroid from the sclera to the point of attachment of the ciliary muscle. With care detach all the choroid and the ring of the ciliary muscle from the sclera, and as the choroid and iris have been previously fixed they conserve their shape perfectly. This procedure has been described by Czermak in 1885.

2. Embedding: The embedding is made in gelatin. The iris is washed in water for 24 hours; then placed in 10 per cent gelatin for 24 hours; then 30 per cent gelatin for 24 hours. Subsequently the iris is placed in a little pasteboard box, filled with 30 per cent gelatin, and is allowed to freeze in the ice box. When the gelatin has hardened, detach the box, and place the gelatin embedded iris in 10 per cent formalin for 24 hours.

3. Sections: The frozen sections are made in the usual manner. The sections must be parallel to the anterior surface of the iris, because in that manner it is possible to obtain all the thickness of the iris in a very few sections. The sections are fastened to the slides by allowing them to dry in position, and then at a particular time, before the drying has gone too far, they are passed rapidly with a glass rod, through celloidin, covering the surface of the sections completely. The sections are then placed in 70 per cent alcohol for fifteen minutes.

4. Depigmentation: The modifications to the primitive Alfieri method are very numerous (Seligmann). We used the following technic: place the section in potassium permanganate, 1:500, for 24 hours. Then rapidly wash in water, and pass into 1 per cent oxalic acid. These operations are repeated as many times as is necessary, until the sections appear white. The time that the sections must remain in the oxalic acid depends upon the previous action of the permanganate. After 24 hours in this agent, 20 minutes in oxalic acid are sufficient. If the sections remain yellow or brown, all the operations must be repeated.

5. Neutralization: When the sections are white, they are passed into a concentrated solution of potassium alum (10 per cent), where they remain until they appear transparent.

6. Staining the nerve fibers: The sections are placed in a solution of 2.5 per cent ferric ammonium sulfate, C. P. (violet crystals) for 2 hours, then rapidly washed in water and passed to the hematoxylin of Heidenhain where they remain 24 hours. After this time the sections are again placed in the solution of ferric ammonium sulfate, C.P., for the differentiation of the medullate nerve fibers from the other parts of the iris. When the differentiation is completed, thoroughly wash in water, then in alcohol 70 per cent, alcohol 95 per cent, carbol xylol, xylol and Canada balsam.

A Case of Adiposis Dolorosa ("Dercum's Disease") with Necropsy. N. C. Foot, Cincinnati.

Abstract. This report deals with a very obese negress of sixty, who weighed over 350 lbs. and was brought to the hospital after a "stroke," unable to answer any questions. She was so fat that physical examination was totally unsatisfactory, beyond establishing the fact that she was covered with great fat-pads that did not involve the head, forearms, hands, legs (below the knee) or feet. No history of painful fat was obtained; had the patient been able to answer questions she might have enlightened us. She died, apparently from uremia, after several epileptiform seizures, less than twenty-four hours after admission.

Necropsy revealed the adipose condition, nephritis and cardiac hypertrophy and dilatation, as the immediate cause of death, and changes in most of the endocrine organs. The pituitary was enlarged and showed adenomatous hyperplasia and sclerosis; the thyroid was also sclerotic and enlarged; the suprarenals were hyperplastic and each presented an adenoma; the ovaries were atrophic and sclerotic; there was persistent thymic tissue, and there was a malignant tumor of the tentorium and a smaller one embracing the stalk of the hypophysis, both of them composed of very primitive cells corresponding in shape and arrangement with those of the dural endothelioma.

The case is discussed from the standpoint of the literature on adiposis dolorosa (Dercum's disease) and it is concluded that this is not a clinical entity, but a syndrome brought about by a variety of causes influencing the hypophysis primarily and the other endocrine organs secondarily, as Cushing has already pointed out.

Filtration with "Oiled" Filters. W. L. Holman, Toronto.

Abstract. While testing filter candles by the method reported by Krock and Holman it was noted that one of the candles showed air bubbles escaping at one spot under a pressure very much below that found in a previous test. This candle, I believe, had accidentally come in contact with a little paraffin on the laboratory table.

A number of filter candles have since been studied and after obtaining estimates of pressure for air leakage, average pore size, capillary pressure, rates of flow and time for cultures of bacteria to pass through, they were artificially treated with a mixture of petrolatum and paraffin oil.

The rate of flow after this treatment was decidedly reduced. Air bubbles also came through under much lower pressure. The Bechhold formula for average pore size would indicate that the pore spaces after they are partly filled with petrolatum and paraffin oil are larger than before treatment. I therefore agree with Mudd in giving more value to the rate of filtration than to such methods for establishing the size of the pore spaces.

A twenty-four hour culture of *B. prodigiosus*, grown at room temperature, was used to test the permeability of the filters and it was found that these bacteria pass through the treated candles in a shorter time and are found in a smaller amount of filtrate than in the untreated candles. These same filters after having the petrolatum mixture removed with xylol showed a more rapid flow of fluid and became less permeable for bacteria. It would appear that the treatment with petrolatum and paraffin oil reduces the absorptive character of the candles and also the size of the filter spaces. It is to be hoped that "oiled" filters may be found useful in reducing the loss by absorption of valuable constituents of filtered materials such as the antitoxins, viruses, enzymes and similar substances.

There is also a chance of error where a filter candle has been accidentally brought in contact with petrolatum or other oily material. Autoclaving does not remove such a substance but rather distributes it over the intergranular surfaces and prolonged treatment with xylol, or other active solvent for petrolatum, is necessary to clean effectively such a candle.

Comparison of the Changes in Blood Sugar Following Injections of Filtrates of B. Paratyphosus B and of Histamine. Maud L. Menten and (by invitation) Helen M. Krugh, Pittsburgh.

Abstract. With single injections of sublethal amounts of histamine, varying degrees of hyperglycemia were obtained, but a fatal hyperglycemia, of a secondary antemortem hyperglycemia similar to those found with paratyphoid B filtrates was not noted. Single lethal injections gave either no change in blood sugar or an immediate hyperglycemia of 250 to 300 mgm. per 100 cc. of blood.

Repeated injections of gradually increasing dosage increased the animal's resistance so that little or no hyperglycemia response to histamine was elicited with each successive injection. A typical anaphylactic rise of blood sugar occurred on injection of histamine in animals previously sensitized to *B. paratyphosus B* filtrates.

Comparison of the Changes in Blood Sugar Produced by Injections of Witte's Peptone and of Filtrates of B. Paratyphosus B. Maud L. Menten and (by invitation) Helen M. Manning, Pittsburgh.

Abstract. Five samples of Witte's peptone, following single injections, caused varying degrees of hyperglycemia but only one of these was capable of producing a hypoglycemia comparable to that obtained with filtrates of *B. paratyphosus B* in rabbits. We were not able to reproduce with peptone, filtrate curves consisting of an initial hyperglycemia followed by an interval lasting from several hours to a week, at the end of which time the animal died with a hyperglycemia. An injection of peptone repeated within forty-eight hours after the first injection gave a fatal hypoglycemia in three to five hours with one sample of peptone. A typical anaphylactic rise of blood sugar was obtained by an injection of Witte's peptone in rabbits previously sensitized to paratyphoid B filtrates.

Some Unusual Findings in the Vermiform Appendix. Alfred Plaut, New York.

Abstract. 1. Encapsulated plant material in serosa of appendix. The patient S. S., 52 years old, Russian, had typhoid fever at the age of 12 and an abscess in the right axilla following it. In the year before admission to the hospital she had different attacks of pain in the right side. Four months before admis-

sion there was an attack of pain in the right lower quadrant, nausea, vomiting and fever (101 F). She was five days in bed.

Physical examination showed tenderness over the gall bladder region and in the right lower quadrant.

At operation a third degree retroversion was found with firm pelvic adhesions. The appendix showed nothing pathologic.

Gross specimen: Thin, slightly hyperemic appendix, 4.5 cm. long. In the serosa near the tip several glassy gray spherical cyst-like formations are found, the size of a millet seed.

Microscopic: The appendix is obliterated. The glassy foci in the serosa are onion-shell formations with plant material in their center. The plant cells are prismatic. They fully resemble cells from wheat seeds. The nuclei of these cells cannot be seen. This foreign material is surrounded by many thick layers of mostly hyalinized connective tissue. Occasionally one solid hyaline mass surrounds the plant material. In some of the nodules large giant cells are found, small ones in others and there are many without giant cells. In the nodules, large and small connective tissue cells are situated between the foreign bodies and the hyaline capsule. All nodules are in the serosa, none in the muscular coat.

Comment. Apparently this plant material came from the intestinal tract. Whether the perforation dates back to the typhoid fever forty years ago or took place only four months ago when the patient had an attack of appendicitis is difficult to decide. Neither explanation is easy to accept. Similar cases are on record observed after perforation of gastric ulcer and after traumatic rupture of cecum. But there is also one instance of peritonitis around plant material in a man 75 years old where the history gave no evidence of perforation.

2. Calcified body in serosa of appendix. A woman 36 years old was operated for a gynecologic trouble. The appendix was retrocecal, adherent to within one inch of its tip and the tip was sharply angulated.

Microscopic. In the serosa a calcified mass is found. It measures 0.3 by 0.15 mm. Surrounded by thin connective tissue it is situated between the serosa itself and the external muscular coat. It is extremely brittle, and pressure upon the coverglass breaks it up into fragments. A structure can be seen under the oil immersion, which seems to be thin layers and some fine openings probably corresponding to stomas. The mass represents a shell or a part of one, which is empty. No inflammation is found in the surroundings.

The patient knew nothing of any previous illness.

3. Eggs of a parasite in wall of appendix. Canadian woman 35 years old; besides her gynecologic history there is a vague statement of appendiceal trouble several years ago. At operation an appendix was removed which was described as short, thick and juicy by the surgeon.

The gross specimen showed nothing particular but microscopically remnants of ova were found in the wall of appendix, partly surrounded by epithelioid cells. One calcified egg has a kind of polar spine. One slide contains a circumscribed fibrous mass which probably represents a scar following total destruction of an ovum. The blood picture of the patient was normal and careful repeated search for ova in feces and urine was negative.

4. Hyalinization of veins in appendix. The patient, 30 years old, white housewife, came to the hospital with symptoms pointing to tubal gestation. Operation revealed a ruptured tube surrounded by brownish bloody material.

Careful microscopic examination shows no signs of pregnancy. The tube wall is inflamed. The blood vessels of tube show nothing particular. A hyperemic appendix 8 cm. long was received with the tube. The periappendicitis which is present is the one usually found after abdominal hemorrhage. The mesoappendix is normal. In the serosa and submucosa of the appendix most of the veins are surrounded by several layers of spindle cells in a similar arrangement as is frequently found around foreign bodies. Inside of the elastica interna hyaline masses are found, partly encircling the whole lumen, partly only one third of it. This material becomes red with eosin, dark orange in the Van Gieson mixture, bright blue with Weigert's fibrin method, red with Russell's method. It gives no reaction for amyloid. The arteries in the appendix are normal. The small veins in the muscular coat are normal too.

The patient has no symptoms of any systemic disease. We cannot explain this disease of the veins of the appendix.

The first two cases indicate that perforation of some part of intestine may take place without stormy symptoms, perhaps even unnoticed.

The third case is probably another instance of Bilharziosis without symptoms.

The last case is submitted in order to get suggestions.

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A STUDY OF HYPERPLASIA OF THE BONE MARROW IN MAN*

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Clinical observations and studies of the peripheral blood have made important contributions to the differentiation and classification of the various types of anemia, but a cloud of mystery still obscures many of the fundamental processes which underlie the diseases of the blood-forming organs. Even when the ultimate cause of an anemia is recognized, the way in which it is produced often remains entirely unknown. The problem of the pathology of the diseases of the blood appears at first sight to be wholly one of physiology, but the trend of recent investigation indicates that in no other field are structure and function more closely interrelated, and it soon becomes apparent that the study of the normal and pathologic physiology of the blood cannot be approached without a simultaneous study of the morphology of the bone marrow. Unfortunately the structure of active human bone marrow, even under normal circumstances, is extremely complex, and in many pathologic conditions the histology is so confused as to defy direct analysis. If, therefore, the diseases of the hematopoietic system are to be satisfactorily interpreted in terms of bone marrow function it would seem worth while to pay attention to the simplest pathologic changes, and the observations to be reported in this paper are the result of a study of the early stages of hyperplasia developing in the atrophic femoral bone marrow in man.

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The fact that comparatively little is known at present about the pathology of the diseases of the blood is largely due to the imperfect and inaccurate information available as to the processes of normal blood formation and destruction, and the many opinions and hypotheses which have been hitherto put forward in this field have contributed little of significance when applied to clinical conditions. Much new light has been thrown on the subject, however, by the recent work of Sabin, Doan and Cunningham [1,2,3,4] and their observations (based on animal experiments, with occasional references to man) appear to be a starting point from which the pathology of the clinical anemias may be gradually built up. Doan recognized that it is almost impossible to determine the structure of a tissue which is as complex as normal animal bone marrow, and in order to simplify the conditions he produced an experimental hypoplasia and then studied the successive stages of the hyperplasia which developed as the marrow returned to normal. In man, however, the conditions are in some ways more favorable to the investigator than they are in the lower animals, for while the active marrow of the vertebrae and flat bones is extremely complex, the marrow of the long bones is normally, at least in greater part, fatty and hypoplastic. Man, therefore, normally provides the necessary hypoplastic and relatively simple bone marrow, and in pathologic conditions one can find the different stages of hyperplasia which correspond to those produced in animal experiments.

Hyperplasia of the bone marrow, in the sense of an increase of cellularity, is a very common type of reaction in human disease and while the more extreme forms, with complete replacement of fat by marrow cells, are usually associated with diseases primarily affecting blood formation, less extensive degrees of hyperplasia are met with in a great variety of conditions, including many acute infections. It would be unwarranted to suggest that the changes which take place in the bone marrow in different acute infections are always similar in character or that any of them necessarily represent early stages of what is found in any particular form of severe anemia, for there is plenty of evidence to indicate that there are many types of bone marrow hyperplasia. It is reasonable, however, to suppose that a study of the simpler hyperplasias of acute infections and the steps in their development from normal hypoplastic marrow may assist the subsequent analysis of more complex pathologic pictures.

The present paper deals directly with the bone marrow of a single case of typhus fever, but the main observations have been confirmed in other cases of the same disease and in other pathologic conditions. This case was selected because of the excellent state of preservation of the tissue and because it shows so many of the stages in the transition from a completely fatty bone marrow to one with a cellularity approaching that of normal human vertebral marrow in the area of a single section. Fig. 1 illustrates, with low magnification, the general character of the material. In passing from the normal to the cellular part of the section it is comparatively easy to trace the successive steps in the development of the hyperplasia. The tissue is an example of relatively pure erythropoiesis and it is fortunately in a state of vascular engorgement in which many of the blood vessels are defined by blood cells almost as clearly as they would be by an artificial injection mass.

The necropsy was performed by Dr. S. B. Wolbach in Poland in 1920, and I am indebted to him for permission to use the material from the case.

The tissue, which was from the femur, was cut in serial sections 6 microns thick, and emphasis must be laid on the fact that many of the observations recorded can only be made by a study of serial sections. In describing the tissue an attempt will be made to consider the various steps in the progressive development of the hyperplasia as nearly as possible in the sequence in which they probably occur. The anatomic nomenclature of Sabin and her collaborators will be followed.

Before proceeding to a description of the pathologic material, brief mention may be made of some of the characteristics of normal human bone marrow from the shaft of the femur. In contrast to the marrow from the epiphyses, which may be moderately cellular and active in a functional sense, the marrow of the shaft of the femur is essentially hypoplastic, inactive and almost without true marrow cells. It consists of large fat cells closely packed together, with occasional elongated, darkly staining nuclei compressed between the fat globules (Fig. 3). As shown much more clearly in the early stages of hyperplasia, these are the nuclei of endothelial cells. The nuclei of the fat cells are not at all prominent and the reticular cells described by Sabin are rare and difficult to distinguish. Another striking feature is the limited degree of vascularity. Small arteries and veins are

present, but the venous sinusoids which are so evident in normal vertebral and in many hyperplastic marrows are narrow and relatively few in number.

It is difficult to determine from the histologic evidence presented by the pathologic tissue under consideration as to what is actually the initial step in the development of hyperplasia in a fatty bone marrow, for two changes are found in areas that are otherwise entirely normal and they apparently take place almost synchronously. One of these is a proliferation of the endothelial cells situated between the fat cells. Instead of the few scattered nuclei which are found in aplastic fatty marrow (Fig. 3), there are large numbers of endothelial nuclei (Fig. 2), and instead of elongated, darkly staining nuclei with little visible protoplasm, there are large, oval, vesicular nuclei with prominent cell bodies lying closely adjacent to the fat globules (Figs. 4 and 5). Doan, Cunningham and Sabin [4] also describe this hyperplasia and hypertrophy of the endothelium.

The second early change is an increase of blood supply, as shown by the appearance of new, wide venous sinusoids, and more particularly by the opening up of collapsed vessels lying between the fat cells. By means of injections with India ink, Doan [3] was able to demonstrate that the narrow, elongated endothelial nuclei lying between the fat cells in atrophic marrow are in fact the nuclei of the endothelial walls of capillaries which are not open to the circulation and which are collapsed by the pressure of the tightly packed fat cells. Drinker [6] also found evidence suggesting the presence of the same vessels. The existence of these "intersinusoidal capillaries" was indicated by the entrance into them of particles of the injection mass, but it is still more clearly shown in the stage of vascular engorgement which is part of the earliest phase of the hyperplasia in the human tissue now under consideration. At this stage, coincident with the appearance of many venous sinusoids, the intersinusoidal capillaries become injected with blood and a vast system of blood vessels, lying between the individual fat cells, is revealed. Fig. 6 illustrates, with low magnification, this early stage in the development of hyperplasia — the large venous sinusoids and the network of intersinusoidal capillaries outlining the fat cells. Some of the fat cells are sectioned at such a level that they are almost completely surrounded by patent capillaries (Fig. 8), and others have an open capillary containing blood cells on one side, and collapsed capillaries

which may be indicated by the long, narrow nuclei of endothelial cells, on the other sides. When blood enters an intersinusoidal capillary and the lumen becomes patent, the walls of the capillary are seen to be attached to the fat cells between which they have been compressed, and as the capillary lumen becomes wider the walls of the capillary remain in contact with the fat cells while the latter become in turn compressed. The structure of the wall of the intersinusoidal capillaries is similar to that of the venous sinusoids, consisting only of a single layer of endothelium, and the fact that, with the development of hyperplasia, venous sinusoids appear where one would only expect to find collapsed capillaries in the atrophic marrow, is evidence that the venous sinusoids are merely capillaries which have become widely open to the blood stream. As Doan, Cunningham and Sabin [4] have stated: "A sinus is a patent intersinusoidal capillary and an intersinusoidal capillary is a collapsed sinus, the state of dilatation or collapse normally depending upon, or at least accompanying, the specific functional capacity shown by the endothelial cells at the moment." The system of intersinusoidal capillaries may be almost completely collapsed in fatty marrow such as that of the normal human femur, and it probably takes little part in the actual nutrition of tissue. This function is carried on by a rather limited number of "transition capillaries" (Doan) which act as intermediary communications between arterioles and venous sinusoids. Fig. 9 shows one of these vessels with a small side branch, lying between fat cells and traversing a widely dilated intersinusoidal capillary.

While the extensive network of intersinusoidal capillaries seems to play no significant part in the nutrition of the bone marrow, Sabin and her associates have shown that it has a most important function in relation to the formation of red blood corpuscles, for the primitive precursors of the erythrocytes apparently arise from the endothelium which forms its walls. This phase of the problem of hyperplasia will be taken up, however, only after the question of the anatomic relationship of the intersinusoidal capillaries to the venous sinusoids has been considered, — a relationship which bears directly on the problem of the delivery of young erythrocytes into the blood stream, and one which can be studied best in the first stage of marrow hyperplasia. The failure of early investigators to recognize this system of intersinusoidal capillaries is probably explained by the

fact that they have usually concerned themselves with the study of animal marrows and the advanced pathologic changes in man, and in such tissues the intersinusoidal capillaries become masked by the enormous numbers of true marrow cells. It is only in the first stage of hyperplasia, when the marrow consists essentially of fat cells and of an open vascular bed, that one can see the relations of the intersinusoidal capillary field to the general circulation.

Doan[3] not only discovered the intersinusoidal capillary bed but he also described the manner in which the capillaries open into the venous sinusoids by means of conical openings in the walls of the sinusoids. These openings are very easily made out in the present case, for many of the capillaries are wide open and filled with red blood cells so that their ramifications can be easily followed. The large venous sinusoids are surrounded by spherical fat cells and between two fat cells one may find a capillary, which has opened up and contains blood, leading toward a venous sinusoid into which it opens by a conical aperture. The sides of the opening are formed by the endothelium of the sinusoid being carried over the convex surfaces of the fat cells so that it becomes continuous with the endothelium of the capillary. The "mouth" of the capillary, where the vessel widens out to enter the sinusoid, frequently contains a number of red blood corpuscles which appear to be about to enter the sinusoid. The upper and lower borders of the conical openings are often defined by the nucleus of an endothelial cell which lies in the horizontal plane, and in favorable places one can make out, by focusing, that the endothelium of the sinusoid bends outward in the direction of the capillary. These openings are illustrated in Figs. 7 and 10. Fig. 11 is a drawing of two openings from intersinusoidal capillaries, one on either side of a fat cell, into a venous sinusoid, and Figs. 12 and 13 are photographs, with high magnification, of each of the openings. These illustrations indicate the character of the openings with unusual clearness, especially when it is considered that they show only a single plane. Convincing evidence, as far as this can be derived from the histology, of the fact that the intersinusoidal capillaries connect with the venous sinusoids by means of conical openings in the walls of the sinusoids, is only to be obtained by focusing and by the examination of serial sections. The study of serial sections also shows that there are innumerable anastomoses between the intersinusoidal capillaries and that the openings into the venous sinusoids

are extremely numerous. In many places the openings appear to come between each pair of fat cells. The mesh of the capillary network is so complex that it unquestionably results in the formation of anastomoses between the venous sinusoids, but whether this intersinusoidal capillary bed is also directly connected with the arterioles is somewhat more uncertain. Large open capillary spaces are often found in close association with arterioles but no openings between the two have been definitely demonstrated. Histologic evidence on this point might well be unobtainable, while the finding by Doan of India ink particles, injected into the arterial circulation, in the collapsed intersinusoidal capillaries suggests they may have reached the capillaries from arterioles, although it is perhaps more probable that the granules entered the capillaries from the venous sinusoids. In the animals injected by Doan, however, the intersinusoidal capillaries were closed and the pressure in the venous sinusoids must have been low. The study of tissue, like that of the present case, in which there are areas with open capillaries and areas with closed capillaries suggests some control over the capillary bed such as is known to exist in other organs. This control over the opening and closing of the capillaries is considered by many to be regulated by the tone of the precapillary arterioles. The venous sinusoids of the bone marrow are formed by a single layer of endothelium and they are without muscular or elastic tissue. It is difficult to see, therefore, how they could regulate a flow of blood out into the capillary bed. Possibly some specific stimulus, acting directly on the cells of the intersinusoidal capillaries or on the arterioles determines their opening as well as their functional state.

Thus far only the earliest phases in the development of hyperplasia of the bone marrow have been described, and it has been shown that in the part of the tissue in which there is least deviation from the normal, the changes consist of an increased vascularization which results largely from the opening of the vast network of intersinusoidal capillaries, and the hypertrophy and hyperplasia of the endothelium of these capillaries.

The second stage in the development of bone marrow hyperplasia is characterized by the appearance of the true marrow cells from which the mature blood cells are derived. As has already been stated, the tissue under consideration shows an unusually pure erythropoiesis and no attention will be paid to the question of leucocyte formation.

The smallest cell groups in which there are only a few very primitive cells may be taken as representing the earliest step toward blood formation. In this tissue there are many areas consisting of endothelial cells and one or more definite megaloblasts, and the striking feature of these cell groups is that the megaloblasts are either attached to the endothelium which forms the intersinusoidal capillaries or they are free, within the intersinusoidal capillaries. Sabin [1] observed the formation of megaloblasts from the capillary endothelium of the living chick blastoderm and watched the megaloblasts drop off the endothelium into the lumen of the capillary. Subsequently she and her associates [4] showed that red cell formation takes place in a similar manner in adult birds and mammals. The histologic evidence derived from the study of early bone marrow hyperplasia in adult man, as illustrated by the present case, is entirely in harmony with the conception that the primitive cells of the erythrocyte series are derived from the endothelium forming the intersinusoidal capillaries, that they separate off into the lumina of these capillaries and develop to maturity within these endothelial-lined spaces. According to this view of erythropoiesis the red blood cells are formed within endothelial-lined spaces which are directly connected with the venous sinusoids and thus within the vascular system. Fig. 14 illustrates the hypertrophy of the endothelium of an intersinusoidal capillary, such as has already been seen in Figs. 4 and 5, and in addition it shows three very early cells of the erythrocyte series, probably megaloblasts, in close association with the endothelium. Fig. 15 is a photograph of a space between four fat cells, such as has been found in the stage of vascularization to be lined by the endothelium of intersinusoidal capillaries, filled by five cells of which three are probably to be classed as endothelial cells and two as megaloblasts, one of the latter being in mitosis. In Fig. 16 there is an endothelial cell, out of focus, lying adjacent to the lower fat cell, and there are two definite megaloblasts in a similar relation to the upper fat cell on the left. At this earliest stage of cellular proliferation, areas may be found in which the fat cells are entirely separated by cords of large cuboidal cells which resemble megaloblasts more than endothelial cells, but some of which are probably erythroblasts. Sabin believes that the megaloblastic stage is usually of short duration and that these cells quickly divide and become what she terms erythroblasts. Fig. 17 is a drawing to illustrate such

an area. Below is an endothelial cell filling the space between two fat cells, and the other spaces which represent intersinusoidal capillaries are largely filled by rows of megaloblasts, erythroblasts and a few more mature cells of the erythrocyte series. Fig. 20 is a photograph of the same area under higher magnification. The spaces between the fat cells, which during the stage of vascular engorgement were seen to consist of capillaries lined with flat endothelium, are in this later stage filled with columns and clumps of cells which are frequently in a state of active proliferation, as shown by the number of mitoses. Double columns of cells may also appear, indicating that the formation of megaloblasts is taking place from the endothelium on both sides of the capillary, and the capillary lumen is usually completely occluded by the cell growth. Where the section cuts the plane between the lower aspect of one fat globule and the upper aspect of the globule below it, one gets, not a column of cells, but a more or less extensive island of primitive cells of the erythrocyte series. At this stage, when active proliferation is in progress, the flat endothelium with its long narrow nuclei is much less apparent than under normal conditions and it seems to be largely replaced by the bigger cuboidal cells which line the capillary spaces. In the areas of early cellular hyperplasia the venous sinusoids are numerous, large and prominent, and the most striking areas of cellular proliferation are usually found in close relation to a venous sinusoid. This is not in harmony with the observation of Doan [4] who found in experimental animals that erythropoiesis was most marked in places in which the circulation was relatively inactive. Not infrequently a row or group of megaloblasts is situated along the outside of a venous sinusoid, between the sinus and an adjacent fat cell; the endothelium of the sinusoid, however, retains its usual flat character with long thin nuclei. It is most probable that the megaloblasts arise from endothelium covering the fat cell rather than from the endothelium of the venous sinusoid which in the case under consideration shows no evidence of taking on any erythropoietic function.

The next stage in the development of the hyperplasia of the marrow is that in which cells of more mature type than the megaloblast and erythroblast make their appearance. Again the process can be best analyzed in the smaller cell groups. After the megaloblasts have become detached from the endothelium of the intersinusoidal

capillaries, they may divide, as shown by mitotic figures, and go through the process of maturation, so that erythroblasts, normoblasts and extruded normoblastic nuclei may be found free in the capillary spaces. Mature erythrocytes may also be present, but it is, of course, impossible to determine whether these cells have developed in the intersinusoidal capillaries or whether they have been brought in from the general circulation. The fact that in the earliest stages of megaloblastic hyperplasia the intersinusoidal capillaries may be occluded by endothelial cells and megaloblasts, and contain very few erythrocytes, suggests that the erythrocytes which reappear in the capillaries with the normoblasts have actually been formed locally. The degree of maturity of the cells varies from field to field and normoblasts are often found grouped together in considerable numbers. These clumps of normoblasts may be found in the center of the capillary space, surrounded by more primitive cells, and they are also likely to lie in close relation to a venous sinusoid. Fig. 18 illustrates this phase of the process. It shows a somewhat larger space between fat cells, filled with a clump of cells containing several erythroblasts, many normoblasts with typical pyknotic nuclei and a few erythrocytes. Reasoning by analogy it may be assumed that this space is a dilated intersinusoidal capillary and the conception is borne out by the presence of the endothelial cell, with elongated nucleus, which lies closely adherent to the upper fat cell.

Up to this point the process of hyperplasia, with the development of cells of the erythrocyte series from the endothelium of the intersinusoidal capillaries and within these capillary spaces, has been comparatively easy to trace, but from now on it becomes more confusing. The cellular areas grow larger and they appear as complex masses of cells without definite structure. All the cell types thus far observed are present and new ones enter into the picture. Endothelial cells, still a part of the capillary wall, may become phagocytes of red blood cells; clasmatocytes with ingested normoblasts or erythrocytes are free in the cell mass; leucocytes of various types have made their appearance; and megalokaryocytes may also be found. Although these larger areas of hyperplasia are without doubt the seat of active erythropoiesis, they may contain comparatively few mature red cells, and these are often grouped near the venous sinusoids, as if they were ready to slip into the circulation. Fig. 19 illustrates a cellular area situated near a large venous sinusoid (on right). The

elongated nucleus of the flat endothelium of the sinusoid may be compared with vesicular nuclei of the hypertrophied endothelial cells in the angle between the two fat cells above and on the left. The latter, in close relation to the fat cell, undoubtedly belong to the endothelium lining the now greatly distended intersinusoidal capillary, and they represent the type of active endothelium from which megaloblasts originate. Free in the cell group are megaloblasts (several near the sinusoid in the upper right corner), erythroblasts, normoblasts, erythrocytes, clasmatocytes which have ingested red blood cells, and a few myelocytes and leucocytes. Fig. 22 is a drawing of a similar group of cells also near two venous sinusoids (upper right and lower left). It is impossible to illustrate these areas satisfactorily with photographs as many of the cells are indistinct at any one level of focusing. The endothelial border of this space is indicated by the flat endothelium along the upper middle fat cell and the hypertrophied endothelial cell lying next to the upper left fat cell. In the center, just below the large sinusoid, is a group of large early cells of the erythrocyte series — megaloblasts and erythroblasts. The shapes of these cells suggest that they were closely adherent to one another and that they have shrunk apart in the process of fixation. The same relationship is seen in the four megaloblasts which lie along the right middle fat cell. This tendency for early megaloblasts to remain closely attached to one another, like the component cells of a tissue, is extremely common and they are often found in rows or double columns. As they develop they seem to lose this adhesiveness and become separate, independent cells, the process being analogous to what Key [5] has described as taking place in the maturation of erythrocytes. Just below the center of the field in Fig. 22 is a megaloblast in mitosis. Fig. 23 is a drawing of a larger cellular area and one which is approximately the size of the cell areas in normal vertebral marrow. Evidences of the endothelium bordering the cell group may be found along the fat cells and the general cytology is similar to that in the smaller cellular areas but it has become complicated through the entrance of more cells of the leucocyte series. It remains, however, much less confused than the picture of normal active marrow as the number of leucocytes is relatively small, the process continuing to be, even in these most hyperplastic areas, one of comparatively pure erythropoiesis.

The bone marrow is enclosed in a rigid container, and cellular

hyperplasia with its associated vascular engorgement can only take place at the expense of something which filled the marrow cavity during the state of aplasia. Fat tissue is peculiarly adapted to being the complementary substance, for fat cells can give up their charge of fat rapidly and can become infinitely smaller without loss of function. Compression, at least within wide limits, does not injure them, for they retain their ability to take up fat again and thus fill any space that results from subsequent retrogression of the hyperplasia. Between marrow cells and fat cells there exists a remarkable reciprocal relationship, in which the former appear to dominate while the latter serve the subsidiary function of filling in any space not occupied by active myeloid tissue. Evidence of this relationship is seen in many experimental conditions in which marrow hyperplasia or aplasia, accompanied by a decrease or increase of fat, arise with extraordinary rapidity. When the cellular areas increase in size there is a simultaneous diminution in the amount of fat. Gradual compression of the fat cells produces a decrease in their size before they disappear completely, and in a very hyperplastic marrow the few remaining fat cells are usually much smaller than the fat cells in an aplastic marrow. In the tissue now under consideration the most highly cellular parts contain about twenty fat cells per high dry field as against approximately thirty per field in the aplastic areas and the individual fat cells are definitely smaller. In a highly cellular marrow, such as the typical marrow of pernicious anemia, the fat often seems to have been completely displaced, but it is certain that the fat cells continue to survive, for as soon as a remission of the disease sets in the hyperplasia of the marrow cells retrogresses and fat is again deposited in the fat cells. The manner in which the bone marrow can alternately take up and give up fat indicates that the fat cells are as constant a part of its structure as the blood vessels and the supporting framework of reticulum. When marrow hyperplasia recedes and the myeloid cells disappear there is not only a new deposition of fat in the fat cells but there is a complete, and often an extraordinarily rapid return to the structure of typical aplastic marrow.

In the highly cellular areas of the marrow, illustrated by Figs. 19, 22 and 23, the venous sinusoids are large, numerous and engorged with blood. They appear to be surrounded by an intact wall of endothelium and there is little to suggest the openings of the inter-

sinusoidal capillaries that are so clearly made out in the areas which show the very earliest stage of hyperplasia. The only possible indication of these openings, indeed, is to be found in the occasional small clumps of mature erythrocytes situated just at the edge of the sinusoid and which may mark points of entrance into the vessel. It is, however, not remarkable that the openings into the venous sinusoids are thus obscured, for the whole structure of the tissue has been completely altered. In the first stage of hyperplasia these openings connected the venous sinusoids with delicate intersinusoidal capillaries, some of which were collapsed and others, more easily distinguished, were filled with mature red blood corpuscles. This was followed by the development of cells of the erythrocyte series within the capillaries, the compression of fat cells and the formation of large cell masses within the intersinusoidal capillaries. In this late stage of hyperplasia the intersinusoidal capillaries have become wide cell beds and their individual limits have become indistinguishable just as a series of brooks which flow through a field lose their identity when the spring floods cause them to swell until they merge and convert the field into a great lake.

In spite of the fact that in a highly cellular marrow it is impossible to observe definite openings between the intracapillary cell areas which form so much of the tissue and the venous sinusoids, the study of the development of hyperplasia certainly indicates that such openings exist and that through them the mature erythrocytes enter the circulation. The process by which the cells pass from their position in the intersinusoidal capillaries into the venous sinusoids is not, however, clear. Histologic evidence shows that the more immature cells are often at the periphery of the capillary and either adherent to the endothelium or adherent to one another so as to form groups or columns, while the more mature cells occupy a central position in the cell mass. It is possible that the latter cells, which have lost their adhesive qualities, are gradually forced towards the venous sinusoid by the pressure of cell growth in the sense of Drinker,[6] and it is also possible that a true circulation through the venous sinusoids, such as appears to be present in the earliest stage of hyperplasia, persists and washes out the mature cells into the veins.

While the tissue which has been described is an example of relatively pure erythropoietic hyperplasia, it also shows a considerable

development of megalokaryocytes. Little is known about the origin of these cells but the fact that in the early phases of hyperplasia they are often found closely adherent to fat cells, in the position occupied by the endothelium of the intersinusoid capillaries, suggests that they, as well as megaloblasts, may arise from endothelium. Fig. 21 is a photograph of a megalokaryocyte in such a position.

Summary and Conclusions

In an attempt to throw light on the pathology of the bone marrow and thus on the fundamental factors which underlie the diseases of the hematopoietic system, the histology of the femoral marrow from a case of typhus fever has been described. This tissue was selected because it is an example of almost pure erythropoiesis and because it shows the various steps in the development from an atrophic to a relatively hyperplastic marrow within the area of a single section. The first changes consist of the appearance of large venous sinusoids, the opening to the circulation of the extensive network of intersinusoidal capillaries, and the hypertrophy and hyperplasia of the endothelium lining the capillaries. At this phase the openings from the intersinusoidal capillaries into the venous sinusoids can be easily detected. In a later stage the precursors of the erythrocytes appear inside the intersinusoidal capillaries, and the histologic picture is consistent with the concept that they are derived from the endothelium of the intersinusoidal capillaries. The earliest islands of marrow cells are composed of a few megaloblasts attached to endothelial cells or free within the capillary spaces. Larger cell islands usually contain more mature types of the erythrocyte series (erythroblasts and normoblasts) and there is a general tendency for the immature cells to be adherent to one another at the periphery of the group and for the mature cells to be free and independent in the center. As the cellular areas increase in size the picture becomes complicated by the appearance of other cell types, and in this advanced stage of hyperplasia the evidence that the erythrocytes develop within capillaries which are in direct communication with the venous sinusoids becomes much more obscure. There are, however, indications that the intracapillary formation of erythrocytes persists even in highly cellular marrows, and this relation suggests the general method by which young red blood cells enter the circulation.

The histology of the type of bone marrow hyperplasia which has been described can be readily analyzed on the basis of the work of Sabin, Doan and Cunningham, and this study of human tissue is offered in support of their conceptions.

I am greatly indebted to Miss Lillian M. Leavitt for the preparation of the serial sections of bone marrow, and to Miss E. Piotti for her beautiful and accurate drawings.

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DESCRIPTION OF PLATES

Plate 91

Fig. 1. General character of material described. Aplastic marrow above; marked hyperplasia below; and intermediary stages in development of hyperplasia in the center. × 50.

Fig. 2. Drawing to illustrate hypertrophy and hyperplasia of endothelium of intersinusoidal capillaries lying between two venous sinusoids. × 500.

Fig. 3. Drawing of normal bone marrow from human femur, showing elongated nuclei of collapsed intersinusoidal capillaries. × 500.

Fig. 4. Hypertrophy of endothelial cell of intersinusoidal capillary. Note relation of endothelial cell to fat cell. × 1000.

Fig. 5. Two hypertrophied endothelial cells of intersinusoidal capillary. × 1000.

Plate 92

Fig. 6. Earliest stage of marrow hyperplasia. Large venous sinusoids and engorgement of intersinusoidal capillaries with red blood cells. × 80.

Fig. 7. Drawing of intersinusoidal capillaries, filled with blood, opening into a venous sinusoid. × 500.

FIG. 8. Drawing of intersinusoidal capillaries, filled with blood, surrounding a fat cell. × 500.

FIG. 9. Drawing of a transitional capillary. Note hypertrophy and hyperplasia of endothelium of intersinusoidal capillaries. × 500.

FIG. 10. Drawing of intersinusoidal capillaries filled with blood and opening into a venous sinusoid. Note nuclei of endothelial cells lying on the floor of the opening. × 500.

PLATE 93

FIG. 11. Drawing of the openings of two intersinusoidal capillaries into a venous sinusoid. Note the incurving of the endothelium of the sinusoid to meet and form the wall of the capillary. The drawing indicates the funnel-shaped character of the openings and the two following photographs confirm this appearance. × 500.

FIG. 12. Photograph of the opening shown on the left in Fig. 11. × 1500.

FIG. 13. Photograph of the opening shown on the right in Fig. 11. × 1500.

FIG. 14. Drawing of hypertrophied endothelium of intersinusoidal capillary and three megaloblasts in close relation to the endothelial cells. × 500.

FIG. 15. Endothelial cells of intersinusoidal capillary, with two megaloblasts. One megaloblast in mitosis. × 1250.

FIG. 16. Two megaloblasts in close association with fat cell (in position occupied by endothelium of intersinusoidal capillary). Endothelial cell, next to a fat cell, shown indistinctly below. × 1000.

PLATE 94

FIG. 17. Drawing of hypertrophied endothelial cells of intersinusoidal capillaries with megaloblasts and erythroblasts developing in the capillaries. × 500.

FIG. 18. Drawing of erythroblasts and normoblasts in intersinusoidal capillary. Note endothelial cell lining the capillary spaces, above. × 500.

FIG. 19. Drawing of larger cell group near a venous sinusoid. Many normoblasts, a few leucocytes, and two clasmatocytes which have phagocytized red cells. × 500.

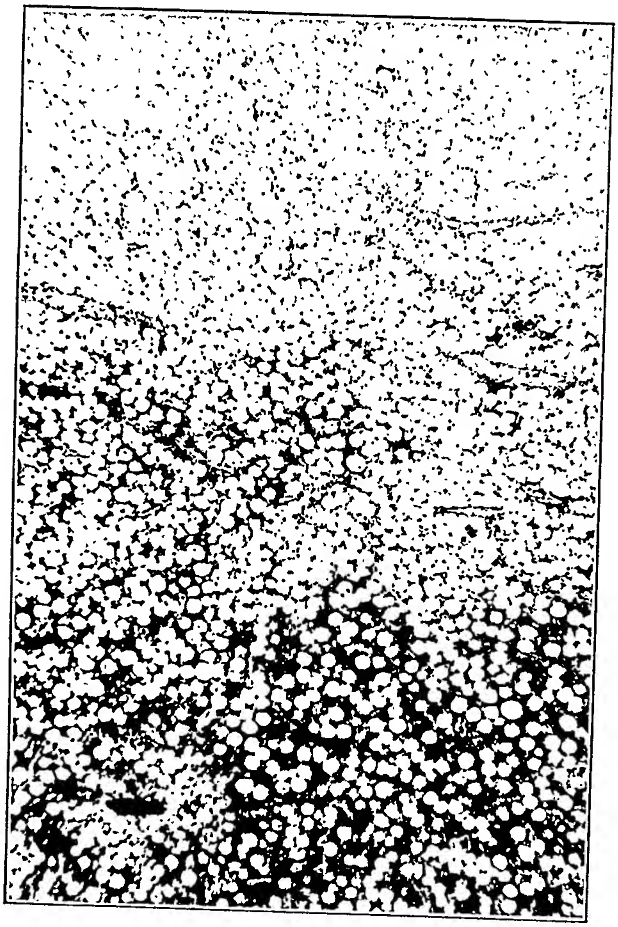
FIG. 20. Photograph of same field as Fig. 17. × 1000.

FIG. 21. Megalokaryocyte lying between fat cells, in position of intersinusoidal capillary. × 1000.

PLATE 95

FIG. 22. Drawing of a group of cells developing between and compressing fat cells. The flat endothelial cell along the upper fat cell and the hypertrophied endothelial cell along the upper left fat cell suggest that the group of cells is inside an intersinusoidal capillary which has become greatly distended. Note megaloblasts, one of which is in mitosis, in center. × 500.

FIG. 23. Drawing of a larger group of cells. In several places elongated or vesicular nuclei of endothelial cells, lying closely attached to fat cells, suggest that the cell island is developing within a space lined with endothelium. Note further decrease in size of fat cells. × 500.



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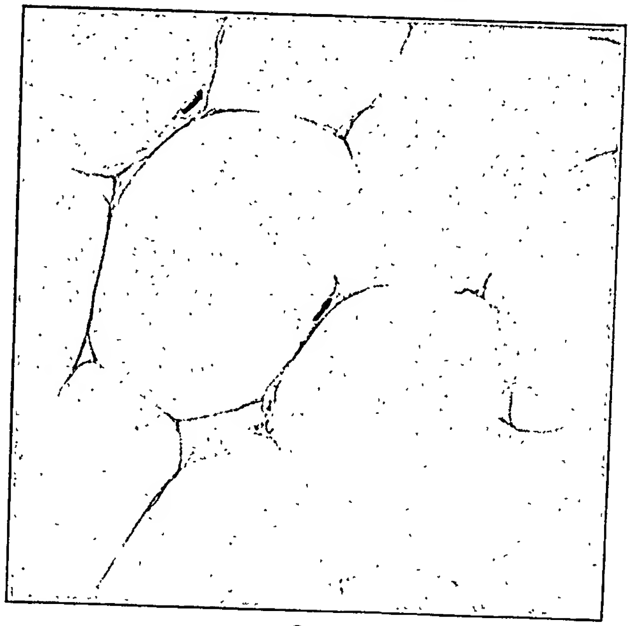

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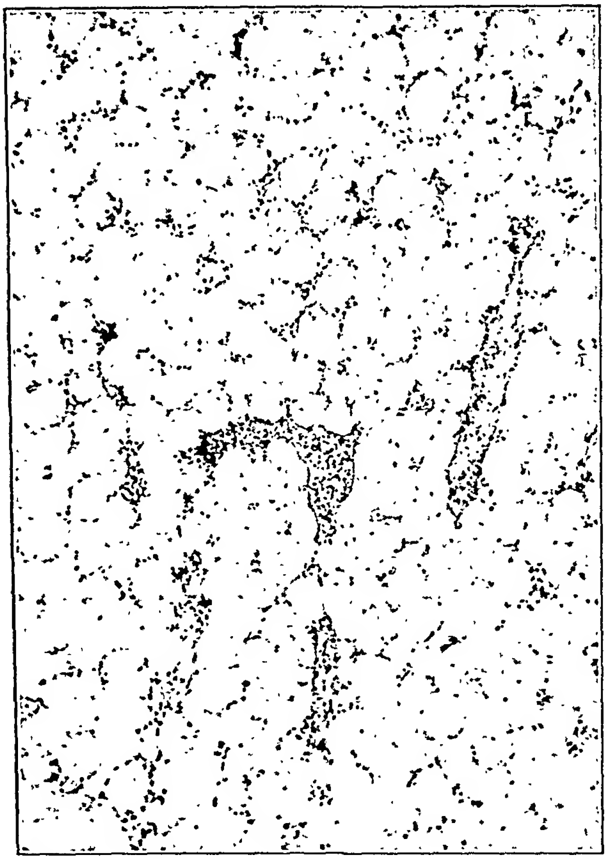
Peabody

Hyperplasia of Bone Marrow in Man



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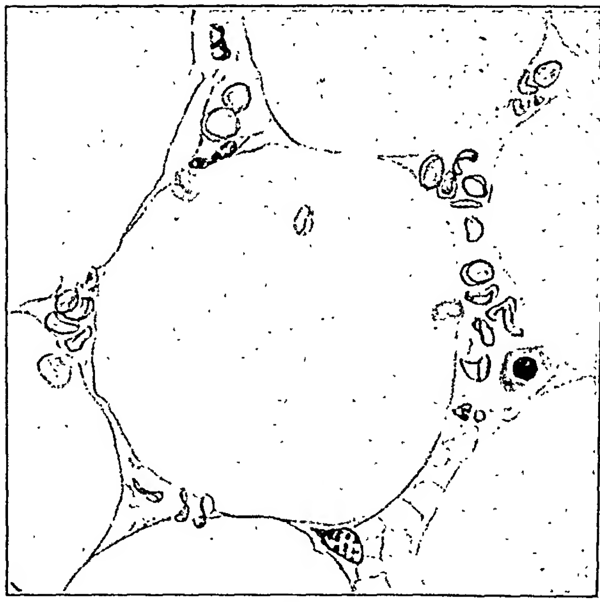

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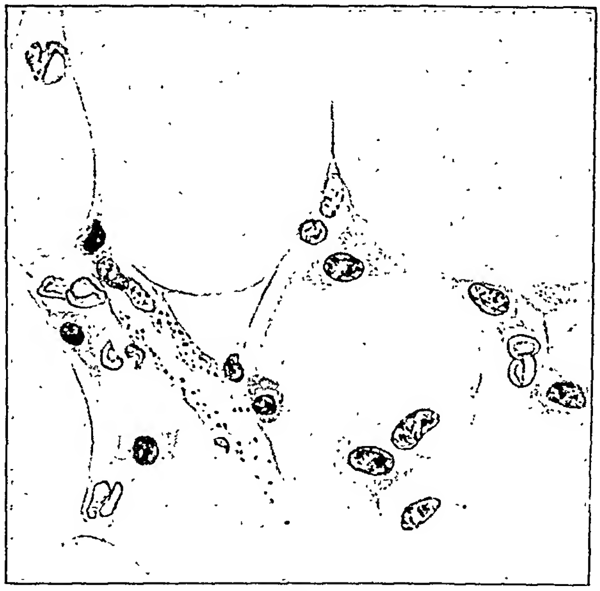

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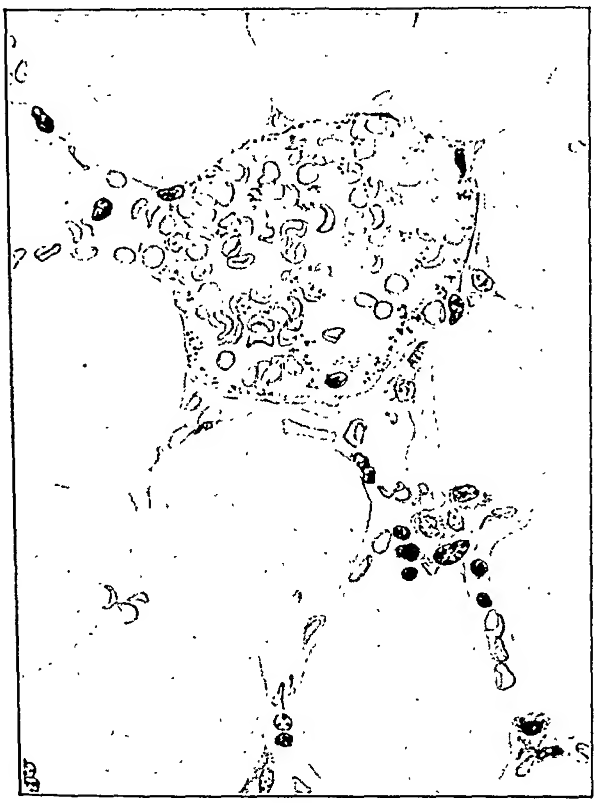

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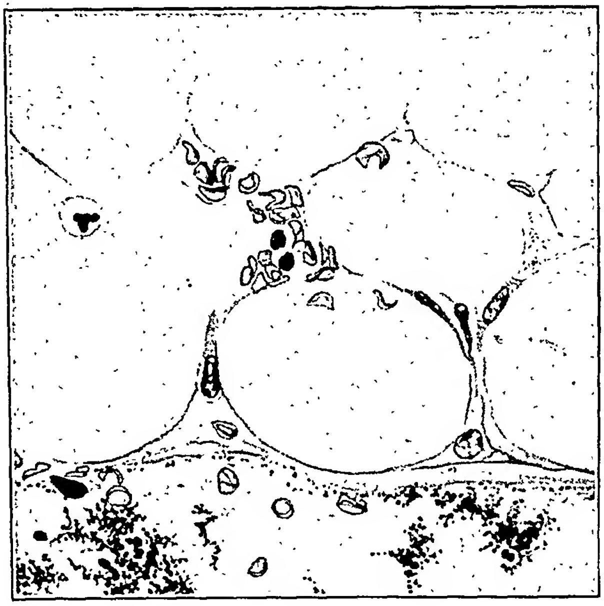
Peabody

Hyperplasia of Bone Marrow in Man



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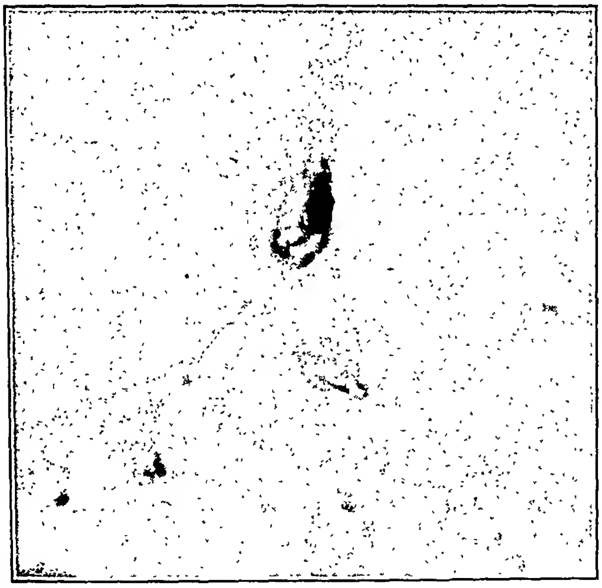

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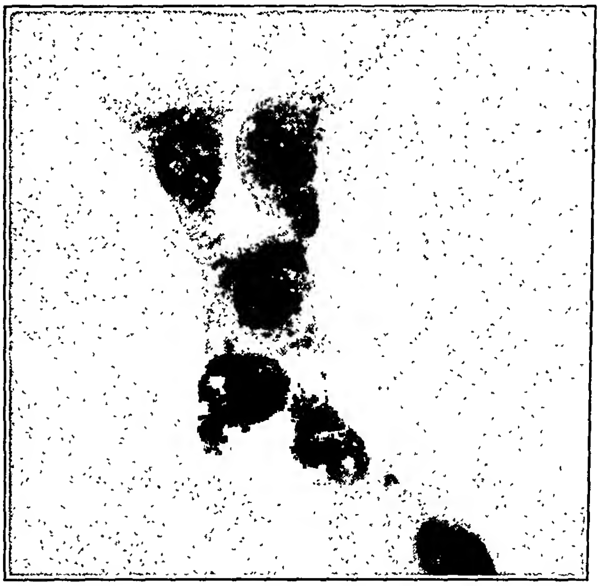

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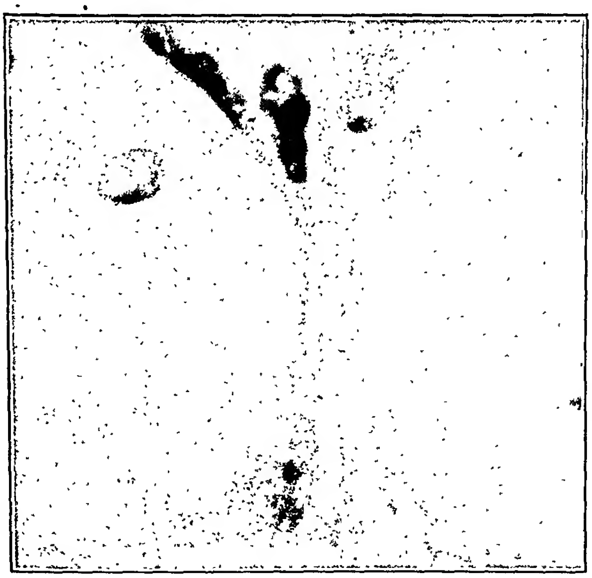

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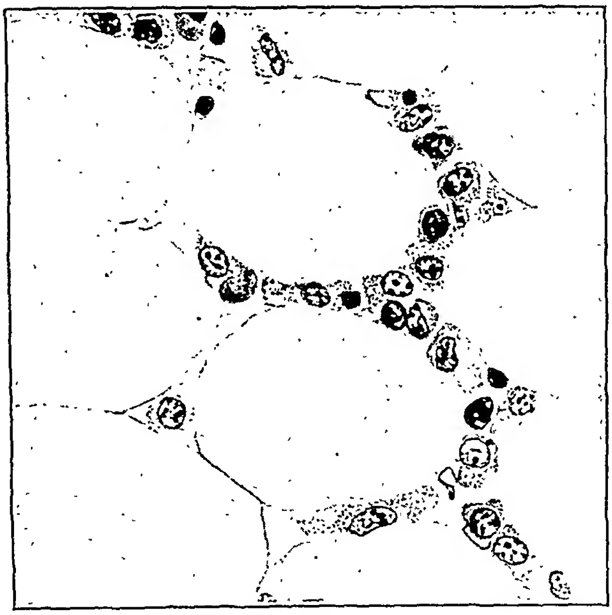
Peabody

Hyperplasia of Bone Marrow in Man



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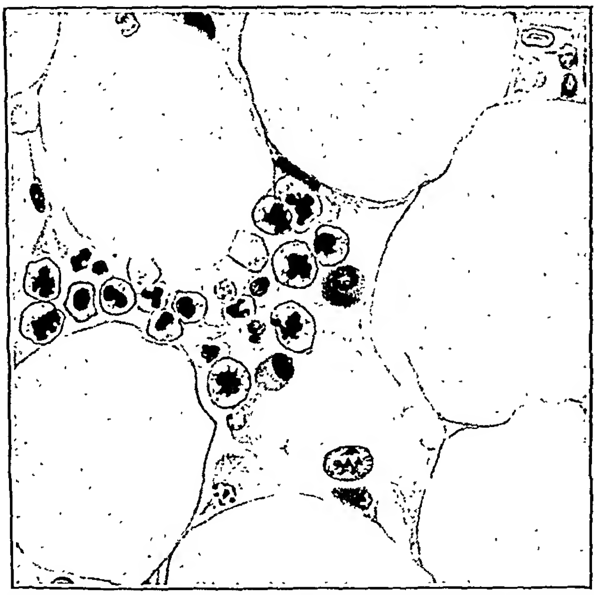

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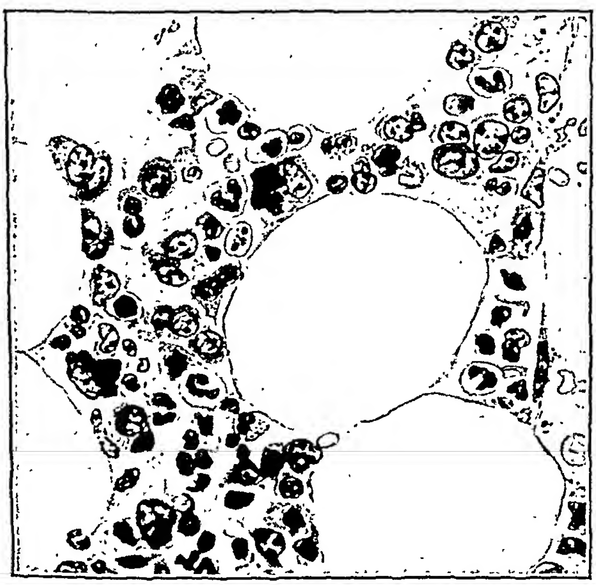

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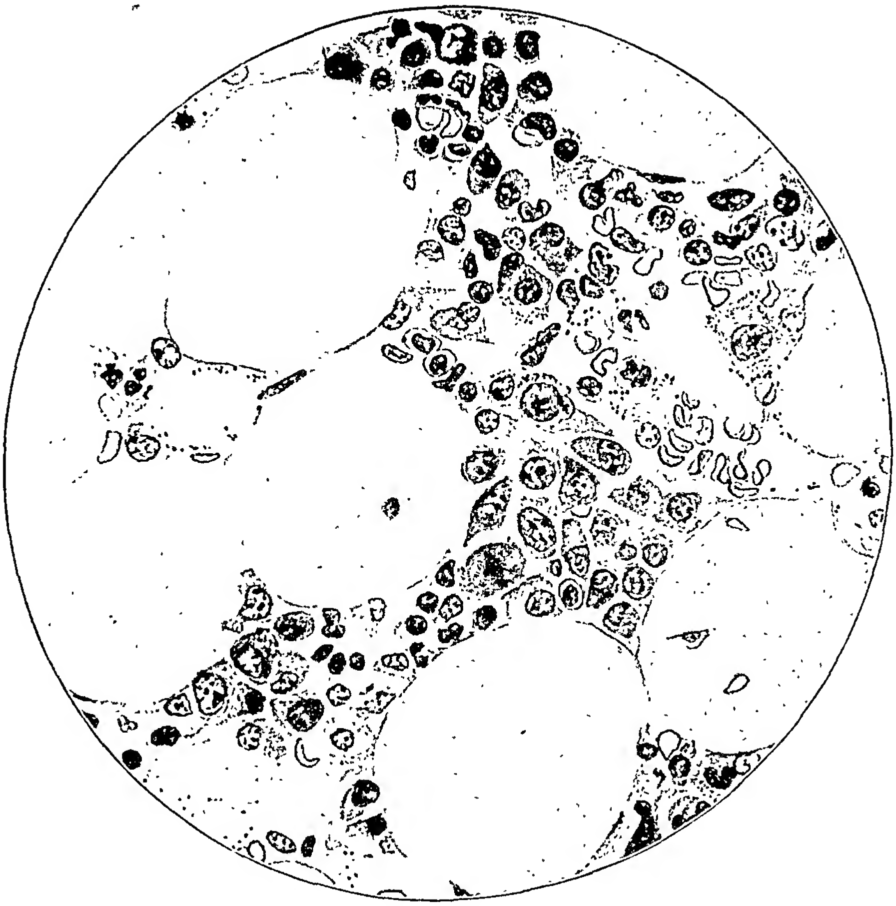
Peabody

Hyperplasia of Bone Marrow in Man



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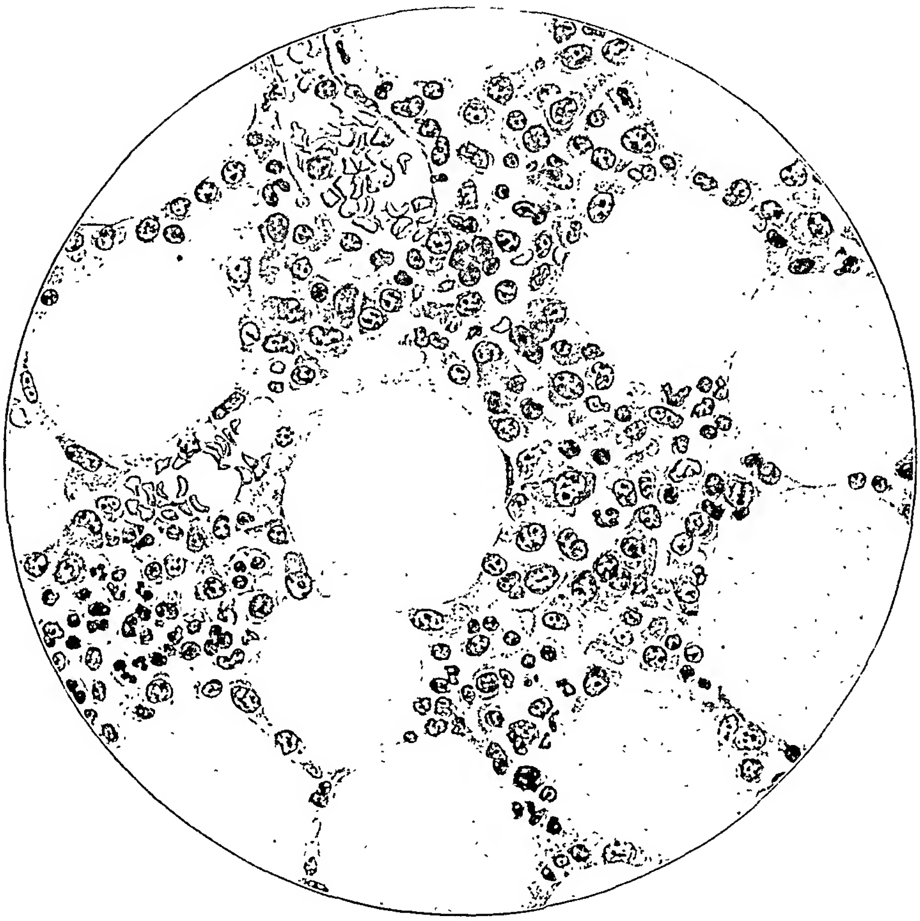

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Peabody

Hyperplasia of Bone Marrow in Man

FIBRIL FORMATION BY HUMAN LUTEIN CELLS *

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Several years ago I happened to observe conspicuous fibrils attached to the large cells of a well preserved and apparently normal corpus luteum. In Zenker-fixed tissue these fibrils were visible, stained bright pink with the Mallory eosin-methylene blue technic; but they were best seen after using his phosphotungstic acid hematoxylin.[1] Demonstration of these fibrils to several persons indicated that their existence was not generally known. Moreover, inquiry failed to disclose any reference to their having been already described. They were not seen again, however, in a succession of rather indifferently preserved corpora lutea studied in routine surgical tissue examinations. Neither was it possible to demonstrate them in the corpora lutea of such laboratory animals as were then immediately available, *i. e.*, rabbits and white rats. But from time to time human corpora lutea have come to hand in which fibrils similar to those first seen could be found. Therefore, it seemed worth while looking for them systematically in available surgical material.

The search has shown them with sufficient frequency to warrant a careful description of these lutein fibrils as a hitherto unnoted normal feature in the development of lutein cells. In fifteen corpora lutea the preservation was good enough so that myoglia and fibroglia were demonstrable in the sections. Lutein fibrils were distinctly seen in eleven of these fifteen corpora. Their presence was doubtful in two others and in two obviously early corpora lutea spuria they were absent entirely. In every one of five corpora lutea vera fibrils were abundant and distinct. These included one normal five months' pregnancy, three ectopic pregnancies and one post-abortion case. Of seven cases of corpora lutea spuria fibrils were well developed in one late case in which shrinkage of the corpus had begun; they were very clear but somewhat less numerous in one fully developed case, while in two others relatively few were seen, and their presence was

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doubtful in a fourth; two early cases showed no fibrils as stated above. Three cases of corpora lutea cystica are included. Fibrils were conspicuous in one, scanty in one and doubtfully present in the third. The cystic corpus in which fibrils were well developed was in the same ovary as the advanced corpus luteum spurium in which fibrils were most evident. It is indicated from these admittedly few observations that the development of fibrils is evidence of full differentiation of the lutein cell. The difficulty of obtaining even one complete series of normal human corpora lutea properly fixed will be readily appreciated.

The fibrils are to be found running in various directions over the surface or within the marginal cytoplasm. For the most part they tend to run with the long axis of the cell but they are unique among other known cell fibrils in that they frequently intersect each other so as to produce a basket-like network about the cell. Some of the fibrils are much heavier than others in the same cell. In certain preparations, also, they are larger on the average than in others. In consequence of the irregular course taken by the fibrils, not all of them appear as long straight lines even when the cell is cut longitudinally. When the cell is cut transversely or more or less obliquely, most of them are seen as short rods, sometimes curved, and as sharply stained dots. In such cases the marginal distribution of the fibrils is particularly evident. Note Figs. 7, 8, 9 and 10. It is, of course, much easier to find cells cut a little obliquely than those sectioned exactly transversely; and since the sections studied are appreciably thick (approximately 7 microns), the dots and short rods often appear to be more or less deeply embedded within a given cell. This is shown in Fig. 6. But careful focusing discloses the fact that they are in all cases strictly peripheral despite such appearances. Where the fibrils intersect there is often a more or less conspicuous thickening. The thickness of these points varies rather strikingly in different cases. In some (Fig. 5) it is very slight, while in others (Figs. 11 and 13) it is extreme. It seems possible that the age of the corpus luteum may be a factor in this. Thus, the best marked examples in the series of cases studied are to be found in a corpus luteum verum in a five months' pregnancy. In this case some cells were provided with networks of very coarse fibrils while comparatively fine fibrils in other cells were at times to be found radiating from large, irregular nodal points. These nodal foci stained some-

times as deeply as the fibrils themselves, although in other cells of the same case they did not stain blue-black with phosphotungstic acid hematoxylin but instead stained a brown-lilac color, similar to but rather deeper than the cytoplasm proper.

In 1915 Corner [2] called attention to deeply stained, often somewhat shrunken, branching cells in the corpus luteum of the sow. He designated them "additional cells of the corpus luteum, type 2" and credited Delestre with having noted them previously. Corner regards their significance as obscure, although in his later paper (1919) [3] he considers them as unaltered, migrated theca interna cells. What surely are corresponding cells are commonly present in human corpora lutea which have attained maximum development. They stain more deeply than the surrounding lutein cells, they are shrunken into an irregularly, richly branching stellate form and their nuclei are either very deeply stained (pyknosis) or exhibit karyolysis. Except for the absence of leucocytes in their vicinity, they possess all the criteria of necrotic cells and I interpret them as such. Their frequency varies in different cases as does also the degree of shrinkage. They occur singly and have not been seen in groups. My material is insufficient to warrant a definite statement as to the origin of these cells. Many of the cells in young corpora lutea spuria are stellate and have dense cytoplasm with well preserved nuclei. Whether or not these are ingrowing theca cells which later undergo necrosis I am not now prepared to say. But running out from the necrotic cells above described, deeply staining fibrils are often to be seen. Fig. 14 illustrates this feature. It is a curious fact that whereas the lutein cell fibrils are susceptible to postmortem changes in the tissue as a whole and are clearly demonstrable only in material preserved soon after operation or very early postmortem, these fibrils seem to persist in isolated, individual necrotic cells. The suggestion is advanced, therefore, that such appearances as are shown in Figs. 16 and 17 owe their origin to further shrinkage of necrotic lutein cells. This may explain the frequent occurrence of such coarse fibrillar networks as are seen in Figs. 11 and 13 in old, fully developed corpora lutea vera.

Lutein cells are generally described as ovoid or polyhedral. Excepting for the presence of marginal fibrils the human lutein cell appears from the study of this material to have no definite cell membrane, and as shown in Fig. 2 the cells not infrequently assume a

broadly stellate form. Associating these observations one is inevitably reminded of the fibroblast. But the lutein cell is always stouter, the stellate form is less evident and the fibrils vary in size in the same and in neighboring cells. Moreover, fibroglia never form the characteristic network to be found in lutein cells. The demonstration of these fibrils cannot, therefore, be cited as evidence in favor of the ancient theory of the purely connective tissue origin of lutein cells. The so-called theca interna cells or theca lutein cells of R. Meyer[4] are generally admitted to be of connective tissue rather than germ cell origin. They are often conspicuous and always to be found in groups peripheral to the mass of lutein cells and its own delicate stroma. They are much smaller than true lutein cells and stain more deeply. I have never seen fibrils in these theca cells although nearby lutein cells showed them sharply stained. My study of human corpora lutea inclines me to the view that the characteristic lutein cell represents a specialized development of zona granulosa cells which are in turn, of course, germinal in origin. It becomes a distinct type of cell.

Lutein fibrils are not easily confused with any other formation in the section. When present they are clear and unmistakable. Their demonstration requires fixation of thin slices of tissue in Zenker's fluid. Their susceptibility to postmortem change has been mentioned. It is further shown by the fact that when a thin slice (1 to 2 mm.) of a corpus luteum was fixed it gave sections in which fibrils stained brilliantly, but in another block of the same corpus 8 mm. thick, fibrils could be seen only dimly in many of the cells in central sections, staining fairly well near the outer edge. Fibrin threads in the central clot, however, stained with equal brilliancy in both preparations. Skeins of fine fibrin threads are often found between the lutein cells in early corpora lutea but there is no confusing these with the lutein fibrils herein described. They are focal, usually near groups of erythrocytes and not attached to the lutein cells. The lutein fibrils occur with equal abundance in all parts of the lutein parenchyma. Lutein fibrils differ essentially from mitochondria in the fact that they are not destroyed by acid fixatives. Moreover, the fibrils are strictly peripheral in the cell, never being found in the central cytoplasm nor arranged about the nucleus as is commonly true of mitochondria. Neither should the lutein fibrils be confused with the extracellular basket-like reticular fibers. The reticulum is com-

paratively coarse, and in Zenker-fixed material stained by Mallory's anilin blue connective tissue method the fibers of reticulum are bright blue in contrast with the clear red fibrils described in this paper.

The function of these interesting fibrils is not determined. As the blood clot in the center of the young corpus luteum is organized, the fibroblasts which in company with capillary sprouts have early made their way inward from the theca interna passing through the metamorphosing zona granulosa, multiply and give rise to a steadily increasing mass of collagen fibrils. For a considerable time, certainly until toward the close of pregnancy in the case of the corpus luteum verum, the lutein cells are provided only with a delicate stroma of reticulum. It is conceivable that the fibrils herein described are supportive in function. In common with other fibrils they may also possess a contractile quality. In all respects so far as studied they seem to belong to the general group of glia fibrils which includes the recognized neuroglia, myoglia and fibroglia. All of these are best demonstrated after chrome-sublimate fixation (Zenker) and when well fixed stain somewhat feebly with eosin but brilliantly with phosphotungstic acid hematoxylin and with Mallory's acid fuchsin stain. All are susceptible to postmortem changes which in certain instances give rise to peculiar alterations in the form of the fibrils before their actual disappearance. All are considered to lie partially or entirely within the cell body of their respective cells but are strictly marginal. Each type of fibril differs in morphologic details from the others and thereby may serve as a means of identifying the type of cell producing it.

It therefore seems proper and may be found useful to designate the fibrils of the lutein cells by an appropriate term. Lest strong objection be raised to combining Latin and Greek roots in the suggestive term *luteoglia*, I would offer as an alternative an euphonious word of purely Greek derivation, *xanthoglia*. This might be freely translated to mean the adhesive fibrils of the yellow cells or lutein cell fibrils.

W. H. Lewis [6] finds no evidence of smooth fibrils and very little of striated fibrils in cells growing in culture and concludes that such appearances as are so well known to histologists are fixation artefacts dependent upon coagulation of the contractile substance in the cytoplasm along lines of tension. Whether or not histologists

generally come to accept this view, it is important for practical purposes to recognize any structural appearance which can regularly be demonstrated in the course of cell or tissue development by the application of a standardized technic. From this standpoint it is clear that xanthoglia are special fibrillary structures demonstrable in the development of human lutein cells.

Summary

Hitherto undescribed fibrils have been demonstrated in the marginal cytoplasm of fully developed human lutein cells. The fibrils tend to form a network enclosing each cell. The term *xanthoglia* is proposed to designate these fibrils.

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DESCRIPTION OF PLATES

All the photomicrographs were taken at the uniform magnification of 650 diameters. The sections are approximately 7 microns thick.

Plate 96

Fig. 1. The large, paler cells to the right are lutein cells cut approximately transversely. The peripheral fibrillar network is shown. The smaller darkly stained cells to the left are the characteristic theca interna cells and are devoid of fibrils.

Figs. 2, 3 and 4 show lutein cells cut more or less longitudinally. The course of the fibrils and their variation in size are clearly seen.

Fig. 5. The surface of a cell in the lower part of the figure has been brought into focus to show the basket-like network which encases the cell. In this instance the network is composed of very delicate fibrils.

Figs. 6, 7 and 8. Various views to show the peripheral distribution of the fibrils about the lutein cells. The cells are sectioned transversely and the fibrils, therefore, appear as dots and short black lines and form a part of the margin of the cells. The apparent inclusion of the fibrils within one of the cells

in Fig. 6 is due to the fact that the fibrils are approaching each other around one pole of the cell. The observer is looking down upon and through a truncated conoid of cytoplasm in which lies the cell nucleus and over the surface of which are spread fibrils.

PLATE 97

FIG. 9. The lutein cells have shrunken away from each other in the preparation of the material. The frequently eccentric position of the nucleus and the peripheral distribution of the branching fibrils can be seen. The photograph was taken from the margin of a corpus luteum. The small, darkly stained cells at the bottom of the figure are theca cells.

FIG. 10. One cell is almost completely surrounded by fibrils. In the center is a surface view of a cell showing a rather coarse fibrillar network.

FIG. 11. A coarse surface network with wide nodal points.

FIGS. 12 and 13. Two views of the same cell taken at different focal levels through one pole. With the nucleus out of focus in Fig. 13 the coarse fibrillar network and thickened intersections can be clearly seen.

FIG. 14. A shrinking, necrotic lutein cell with persistent fibrils.

FIGS. 15 and 16. Different focal levels of the same cell. An irregular, deeply stained body is seen in sharp focus on the surface of the cell in Fig. 16. Fibrils radiating from the body can be followed over the surface of the cell in both figures.

FIG. 17. The bodies seen at their intersection are sometimes more lightly stained than the fibrils.

Figs. 1, 3, 6, 7, 8, 10, 11, 12, 13, 15, 16 and 17 are from a corpus luteum in a case of a five months' intra-uterine pregnancy.

Figs. 2, 4 and 14 are from a corpus luteum in a case of tubal pregnancy.

Fig. 5 is from a corpus luteum in a case of ectopic pregnancy with secondary ovarian implantation.

Fig. 9 is from a corpus luteum spurium.

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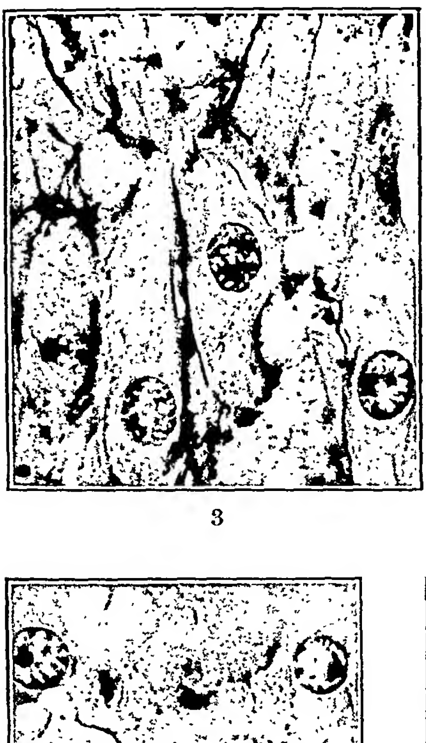

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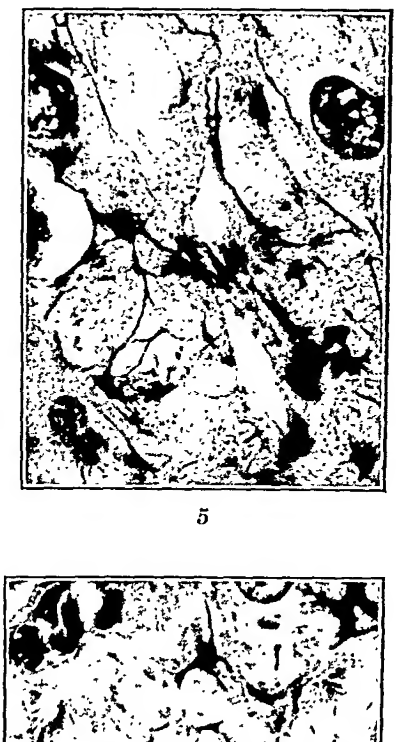

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Fibril Formation by Human Lutein Cells

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Fibril Formation by Human Lutein Cells

NECROSIS OF THE MALPIGHIAN BODIES OF THE SPLEEN *

Norbert Enzer, M.D.

(*From the Snydacker Fund of the Michael Reese Hospital and the Nelson Morris Institute for Medical Research, Chicago, Ill.*)

In 1921 Feitis,[1] under the term "*Fleckmilz*," described a condition of the spleen of which he was unable to find any report in the literature. The cut surface of the organ was studded with miliary, linseed-sized and even larger, round, oval and irregular gray-white nodules which stood out rather sharply against the reddish brown background. Most of them were small and circumscribed, but many coalesced to form larger areas. The picture closely resembled that of disseminated caseous bronchopneumonia. Microscopic examination showed that the lesions were areas of necrosis due to occlusion and thrombosis of the medium and smaller sized arteries. Feitis reported two examples of the condition, both in cases of chronic cardiovascular-renal disease with marked thickening and hyalinization of the media and intima of the smaller arteries of the spleen. Geipel[2] and Matthias[3] in 1924 each reported a case of eclampsia in which the spleen presented areas of necrosis and conformed grossly to the condition described by Feitis. In these cases fibrinous thrombi were found in the small splenic arteries. Two additional cases of arteriosclerosis with multiple necroses of the spleen were reported by Meuret[4] in 1925. In the same year Wilton[5] reported a case of acute upper respiratory infection with involvement of the frontal sinus; death followed pneumonia and pleurisy. At necropsy the pituitary gland was necrotic and the spleen was much enlarged and presented the characteristic picture of "*Fleckmilz*."

Seven cases have therefore been reported and these fall into two groups. On the one hand are those of Feitis and Meuret, in which the lesion was associated with arteriosclerosis, and on the other are those of Geipel, Matthias, and Wilton, in which the underlying condition was one of toxemia or infection. The authors who have followed Feitis have not called attention to any previously reported cases, so that it would appear that the condition is an unusual one.

* Received for publication July 16, 1926.

That it had previously gone unrecognized is inconceivable, since the microscopic picture presented by the condition is so striking as at once to attract attention, although the gross appearance may be deceptive and may simulate miliary tuberculosis or even simple hypertrophy of the Malpighian bodies. An additional case of multiple necrosis of the spleen, in a disease in which the process has not hitherto occurred and differing in pathogenesis from the previously reported examples, is therefore worthy of record.

Case Report

The instance reported here was found in the spleen of Mrs. M. F., 59 years of age, admitted to the female medical service of the Michael Reese Hospital on Sept. 22, 1925. Her complaints were weakness, loss of appetite, loss of weight and numbness over the body, fingers and toes. These symptoms had begun two years previously, but she had been "run down" for eight years. Four weeks before admission she had had several blood transfusions in another hospital. Neither in that hospital nor during her stay in the Michael Reese Hospital did she receive any roentgen-ray treatment.

Physical examination revealed an elderly, emaciated woman, rather drowsy, who dozed off to sleep frequently but was easily aroused. The skin was lemon-yellow in color and the conjunctivae were slightly yellow. A few petechial spots were present over the sternum and abdominal wall and these later increased in number and became more widely distributed. The tongue was smooth, brownish in color and sore. The mucous membranes of the mouth and lips were pale. The heart, lungs and abdomen were negative. On admission the temperature was 102.8 F and ran an irregular course between 98.6 and 103.6 F, with a terminal temperature of 105.3 F. The pulse was small, regular and varied from 100 to 130 per minute. Systolic blood pressure was 100, diastolic 60. The blood Wassermann reaction was negative. Blood culture was negative. Occult blood was present on two occasions in the stools. The urine contained a trace of albumin and a few erythrocytes, leucocytes and epithelial cells. The red blood cell count on admission was 2,200,000 per cu. mm. with 60 per cent hemoglobin. The leucocyte count was 2,300 per cu. mm. with 30 per cent neutrophiles, 65 per cent small mononuclears and 5 per cent transitionals. In the stained smear poikilocytosis, anisocytosis, polychromatophilia and nucleated red

blood cells were found. On one occasion two myelocytes were present. Five days after admission the erythrocyte count was 1,850,000 per cu. mm. with 40 per cent hemoglobin. A clinical diagnosis of pernicious anemia was made. The condition became progressively worse, stupor developed and the patient died on Oct. 13, 1925, three weeks after admission.

At necropsy, performed eighteen hours after death by Doctor William Bloom, the body was that of an aged, emaciated female, with a deep lemon-yellow color to the skin and conjunctivae. Numerous petechial spots averaging the size of a common pinhead were present over the anterior surface of the chest and abdomen. The subcutaneous fat was colored a deep yellow. The heart was small and flabby, and the myocardium pale. The aorta showed small localized areas of intimal sclerosis. Patchy bronchopneumonia was present in both lower lobes. Desquamation and superficial ulceration of the lower portion of the mucosa of the esophagus and atrophy of the mucosa of the stomach were found. Numerous small, petechial hemorrhages were seen beneath the mucosa of the colon. The mesenteric lymph nodes were reddened but not enlarged. The liver was dark brown and small, measuring 22 × 15 × 18 cm. The spleen was free from adhesions, measured 9 × 5 × 3 cm., was soft, dark grayish blue in color, and had a slightly wrinkled capsule. On the cut surface the Malpighian corpuscles appeared prominent and gray, standing out against the red-brown of the surrounding tissue. The kidneys were small; the capsules stripped easily, leaving a finely granular surface. On cut section the cortex was thin, but the markings were distinct. The posterior wall of the bladder was injected and contained large, varicosed, distended veins. Two areas of necrosis each about 1.5 cm. in diameter were present about the trigone. The bone marrow of the ribs and femur was bright red and soft. The blood was thin and watery.

Microscopic Examination

The microscopic picture of the bone marrow is one of cellular hyperplasia with numerous nucleated red cells, normoblasts and myelocytes.

The microscopic examination of the spleen yielded a surprise. The routine sections stained by hematoxylin and eosin contain numerous areas of necrosis, each one involving a Malpighian cor-

puscle. This explains their prominence in the gross specimen, and reëxamination of the formalin-fixed tissue confirmed this. In the latter, irregularly shaped small areas of cloudy gray could now be seen scattered throughout the parenchyma, resembling at first glance caseating tubercles.

The necrosis involves a Malpighian body in every instance, sometimes completely destroying its structure, but an artery can be found in practically every one, either centrally or peripherally placed (Fig. 1). The amount of necrosis is not uniform. Some are completely necrotized, others only partially, and in the latter the necrotic process affects the periphery of the corpuscles. Their character is quite uniform, differing only in the degree of necrosis, although in most of them the necrotic process is advanced and complete.

In sections stained by hematoxylin and eosin, the necrotic Malpighian bodies are violet and have a structureless appearance. The groundwork is amorphous and looks coagulated, and scattered in it are fragmented cells and nuclear granules (Fig. 2). The outer margin of the area is more or less sharp. The surrounding pulp stains a deep pink and is very cellular. The tissue spaces are filled with cells, among which are large numbers of mononuclears and pulp cells in addition to the red corpuscles. In the sinuses, immediately about the necrotic areas, are large, pale staining, spheroidal and irregularly shaped cells (Fig. 3) having a granular cytoplasm and large, eccentric nuclei, the chromatin of which is granular and possesses a reticular network. These cells fill up the sinuses, in some places completely obstructing them. They are phagocytic, for in them can be seen broken blood cells and nuclear fragments; in some, well preserved erythrocytes are present.

These macrophages arise from the endothelium of the sinuses. In the pulp and in the incompletely destroyed corpuscles they may be seen budding from the sinus endothelium into the circulation. Since the endothelium of the splenic sinuses differs from the general blood vascular endothelium in its ability to store vital dyes and is therefore considered to be an important part of the reticulo-endothelial system, these swollen phagocytic cells may be presumed to be reticulo-endothelial in origin.

While their presence is more marked around the necrotic areas, they are to be seen also within these areas; in the corpuscles which are not completely necrotized, the cells are present immediately

around the central artery and in the sinuses. Here they are not so large, the nuclei are more distinct and stain deeply, the cytoplasm is clearer, and their phagocytic properties are not so evident. Where these cells are very numerous they are crowded together and distorted, and in the necrotic areas they may be seen in all stages of disintegration. In regions farther removed from the necrotic areas, large cells the size of giant cells are present in the sinuses, which are free of blood. The cytoplasm of the larger cells is granular and their nuclei may be multilobed. Polymorphonuclear cells are conspicuous by their absence.

Mallory's aniline blue stain demonstrates very clearly the reticular connective tissue and vascular walls. These all stain a bright blue; in the areas of necrosis the connective tissue framework is coarser, enmeshing the necrotic cells. In this stain the large endothelial cells stand out rather prominently, their pale blue color forming a good contrast to the red and orange of the other cells.

In a combination stain of Weigert's iron hematoxylin and Van Gieson's connective tissue stain, the vessels and connective tissue elements are pink and the cellular elements yellow with black nuclei. In the larger vessels the intima is irregular and wavy and the media is thickened and hyaline, but nowhere is there obliteration of the lumen or thrombus formation.

Mallory's phosphotungstic acid hematoxylin demonstrates the connective tissue and reticulum in the necrotic areas as rather coarse, reddish violet strands forming an irregular, but somewhat circularly arranged framework. The individual strands are much thicker and coarser than those in the pulp. At the periphery of the necrotic follicles is a narrow, coarsely meshed zone which stains deep blue. The staining reaction is that of fibrin, but the material is unusually coarse. It does not stain by the Weigert fibrin method and is apparently swollen, partly degenerated reticulum. The arteries of the Malpighian bodies are exceptionally distinct in the phosphotungstic acid hematoxylin preparations. Their lumina are patent and there is no thrombosis in any of them.

Discussion

In the two cases originally described by Feitis, the arteries of the spleen were thickened as part of the generalized arteriosclerotic process. In those of the Malpighian bodies, the lumen was de-

creased in diameter and was occluded by recent thrombosis. When the necrosis of the follicles was not complete, it involved the center or periarterial zone primarily, the portion farthest from the vessel being preserved. Feitis considered the process one of anemic infarction, which differed from the usual type of infarction of the spleen in that the foci of necrosis were multiple. This necrosis occurred as the result of occlusion of the smaller arteries, namely, those of the Malpighian bodies, rather than of those of larger size, as is usually the case in the more extensive infarcts which occur singly or in small numbers. Meuret noted the same changes in his two cases, which were also associated with generalized arteriosclerosis, and accepted Feitis' explanation of the necrosis.

In the two cases of eclampsia, with multiple necroses of the spleen, reported by Geipel and by Matthias, the microscopic picture in general was the same as that described by Feitis and by Meuret. Arteriosclerosis, however, was not a factor in the occlusion of the small arteries, which in these cases was also the immediate agent in the necrosis. The vessels were occluded by fresh fibrinous thrombi. The vascular endothelium had proliferated, which process and the thrombosis were ascribed to the action of a toxin. In Wilton's case of pneumonia with multiple splenic necroses, the same condition of endothelial proliferation and recent thrombosis was present, and in this case also the action of a toxin was held to be an important factor in the pathogenesis of the lesion.

In the seven previously reported cases the necrosis of the Malpighian bodies was arterial in origin. In the case here reported the arteries were not obliterated by arteriosclerosis and there was no thrombosis as the result of an acute infectious process. Furthermore, when necrosis was incomplete it spared that portion of the follicle immediately about the artery. The most striking feature of our case was the marked proliferation of the sinus endothelium. This process was generalized throughout the spleen, and was present in the red pulp as well as in the Malpighian bodies. Erythrophagocytosis by these cells was present in greater degree than one usually sees in pernicious anemia. The cellular proliferation is believed to have been a part of the anemia, and only accidentally, because of the peculiarities of the circulation within the spleen, to have been a factor in the necrosis.

Why the necrosis in this case should have begun in the peripheral

portion of the Malpighian bodies and should have been limited to the bodies requires explanation. Although the swollen macrophages are present throughout the spleen, they are most numerous in the sinusoids at the periphery of the follicles. It is only in this situation that the cells are numerous enough completely to fill the sinuses and to obstruct the circulation through them. Possibilities which present themselves are that in this situation the cells were formed in greater number, or that they were aggregated here by the peculiarities of the circulation within the spleen.

One of the last pieces of work upon which Thoma [6] was engaged before his death, and the results of which were published after his death, was a reinvestigation of the circulation of the spleen. He claimed that it is impossible to effect a complete injection of the spleen from the arterial side. If a thin injection mass with finely divided matter in suspension was used, part of the material passed through lacunae in the arterial portions of the sinusoids, the mass becoming thickened and remaining in the arterial sinusoids. If a thicker mass was used it filled the arterial sinuses but did not pass into the venous channels. By means of simultaneous arterial and venous injections with a mass of proper consistence injected under carefully controlled pressure, Thoma claimed that he was able to effect a complete injection of the sinusoidal system of the spleen. According to the results obtained by this method, the arteries follow the trabeculae and pass to the Malpighian bodies. Here each artery breaks up into a number of arterioles which traverse the follicle. At the periphery of the latter the arterioles bend back sharply upon themselves and become suddenly transformed into wide, thin-walled arterial channels which Thoma termed ampullae. Some of the arterioles pass into the pulp and here also become transformed into ampullae, but the latter are most numerous and largest immediately about the Malpighian bodies, where they form a zone of closely placed blood spaces (Fig. 4). The arterial ampullae pass over into the venous sinusoids, but the two are partly separated by a constriction which holds back the arterial injection mass (Fig. 5).

If Thoma's view of the circulation within the spleen is correct, then both the possibilities mentioned above in explanation of the aggregation of the proliferated endothelial cells in the peripheral sinuses of the follicles may have been operative. Since it is in this region that the ampullae are widest and most numerous, endothelial

proliferation would lead to a greater formation of cells here than elsewhere. In addition, the constriction between the arterial ampullae and the venous sinusoids would tend to collect within the former the cells which are formed throughout the Malpighian body and brought to the region of the constriction by the circulation. The combination of greater cellular proliferation in the ampullae and the aggregation of the cells in the latter by the circulation would cause such a filling of the peripheral vascular channels as obtained in this case. Necrosis of the Malpighian bodies due to filling of the sinusoids by proliferated cells is analogous to the focal necrosis of the spleen and liver in typhoid fever from occlusion of the sinusoids by endothelial leucocytes, as described by Mallory.[7]

Summary

The literature contains seven cases of necrosis of the Malpighian bodies of the spleen, the first two having been reported by Feitis in 1921.

In four of these cases the underlying condition was arteriosclerosis, and necrosis was due to thickening of the walls of the arteries of the follicles with terminal thrombosis. In the remaining cases, two of eclampsia and one of pneumonia, the cause of the necrosis was also arterial occlusion, but in these the interference with the circulation was due to proliferation of the arterial endothelium and to thrombosis, both supposedly the result of the action of toxins.

An additional example of the condition, in a case of pernicious anemia, is here reported. Necrosis was limited to the Malpighian bodies, all of which were partially or completely involved in the process. The arteries of the bodies were not occluded. When the necrosis was incomplete it involved the peripheral zone and spared the tissue immediately about the artery.

The sinus endothelium was proliferated and swollen throughout the spleen, and exhibited a marked degree of erythrophagocytosis. The endothelial proliferation is believed to have been a phenomenon of the anemia.

The swollen macrophages were especially numerous in the peripheral sinuses of the Malpighian bodies, being here so crowded as to interfere with the circulation of the blood. Their aggregation in this region is held to have been due to the peculiarity of the vascular structure and of the circulation of the spleen.

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DESCRIPTION OF PLATES

Plate 98

Fig. 1. A group of necrotic Malpighian bodies. To the left of the middle of the field are two with a narrow non-necrotic zone about the artery. Hematoxylin and eosin. × 60.

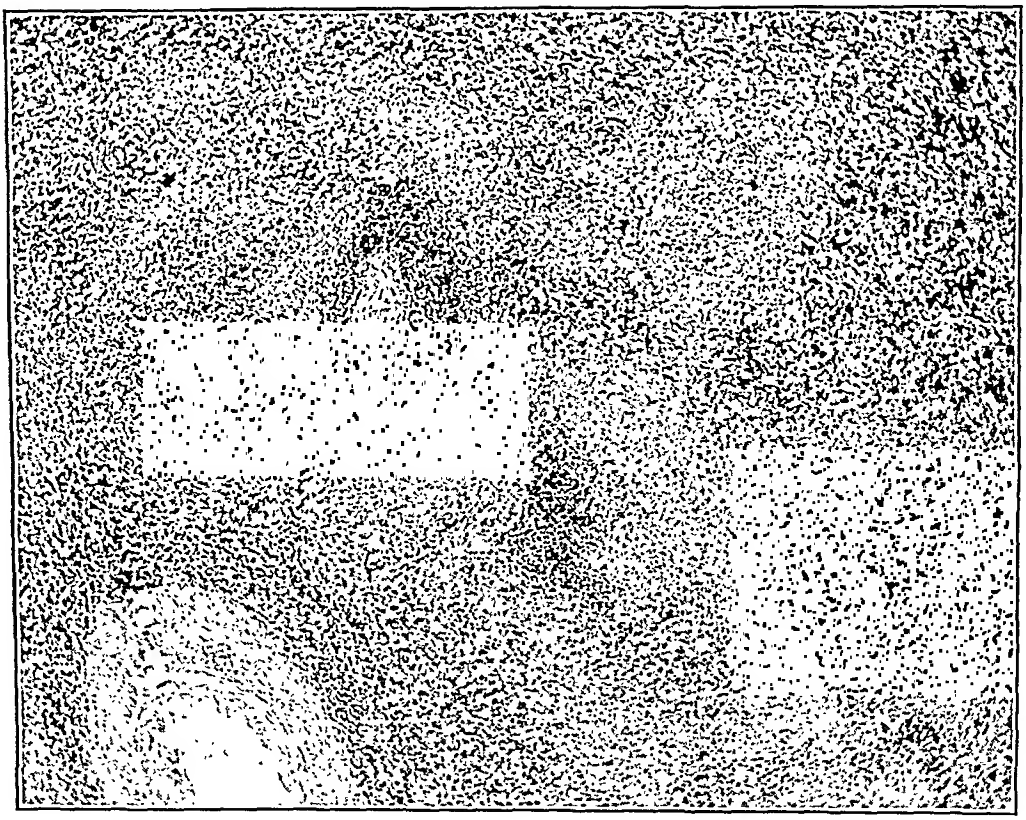
Fig. 2. Karyorrhexis in a necrotic Malpighian body. The artery is not occluded. Hematoxylin and eosin. × 325.

Plate 99

Fig. 3. Swollen reticulo-endothelial cells within the sinusoids at the periphery of a necrotic Malpighian body. Mallory's aniline blue connective tissue stain. × 560.

Fig. 4. Ampullae about the periphery of a Malpighian body; arterial injection of spleen of dog (Thoma).

Fig. 5. Terminal arteriole, with its ampullae (black) and venous sinusoids (gray); combined venous and arterial injection of spleen of dog (Thoma).



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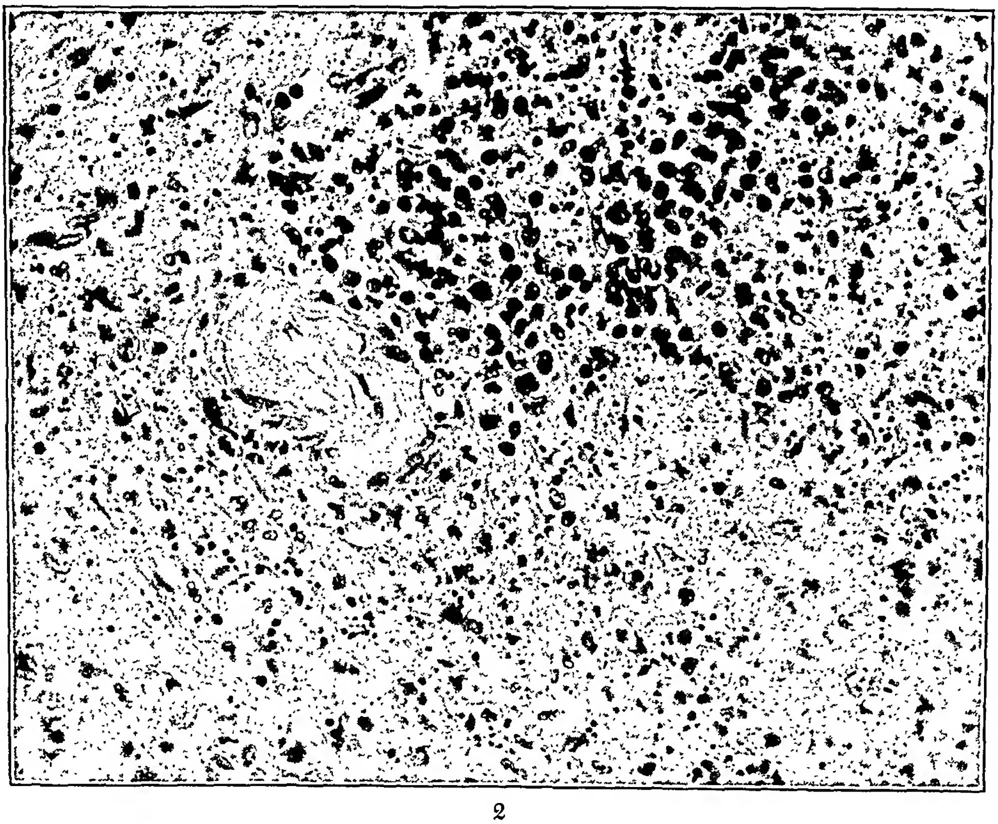

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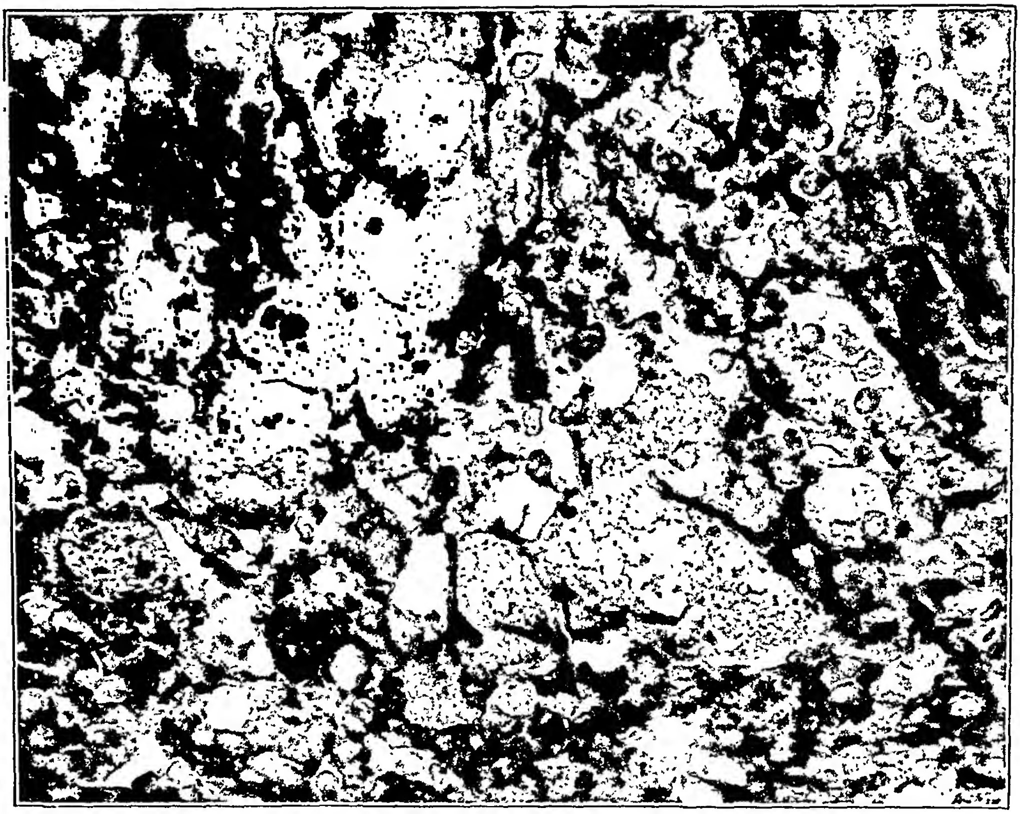

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Enzer Necrosis of Malpighian Bodies of Spleen

HETEROTRANSPLANTATION OF CARTILAGE AND FAT TISSUE AND THE REACTION AGAINST HETEROTRANSPLANTS IN GENERAL

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In former investigations we studied heterotransplantation of skin,[1] thyroid [2] and kidney [3]; we also made some experiments in which blood clots [4] were heterotransplanted. Certain conclusions were at that time reached as to the action of hetero-toxins on the transplanted tissues and the mode of reaction of the host against the strange cells. There were, however, certain points, such as the behavior of the lymphocytes in heterotransplantations, which needed further analysis in order to arrive at a definite understanding of the factors active under these conditions. For this purpose we decided to extend these investigations to a tissue which, in our previous studies on homoiotransplantation, we found more resistant to injurious influences, namely, cartilage. Such a resistant tissue, we believed, might be especially suitable for the analysis of the reaction occurring in heterotransplantation. With the cartilage was transplanted the surrounding fat tissue. In conclusion we shall correlate the principal facts brought out in our various studies of heterotransplantation and thus arrive at a more complete analysis of the agencies active under these circumstances.

Five series of heterotransplantations of xiphoid cartilage were made. In the first and second one we transplanted rabbit cartilage to subcutaneous, ventrally situated pockets in the guinea-pig; in the third series we again used rabbit cartilage, but transferred it to subcutaneous, dorsal pockets in the rat, except in a few cases when we used ventral pockets. In the fourth series guinea-pig xiphoid cartilage was transplanted to dorsal pockets in the rat, and in the fifth series the reverse transplantations were carried out, that is, from the rat to ventral, subcutaneous pockets in the guinea-pig.

We shall discuss (A) the effect of heterotransplantation on the

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life of the transplanted tissue and on its regenerative power; (B) the reaction of the host connective tissue against the transplant; and (C) the behavior of the polymorphonuclear leucocytes and lymphocytes towards the transplanted tissue.

A. The Duration of Life and the Regenerative Power of the Transplanted Tissue

We have to consider in this connection the cartilage, the perichondrium and the surrounding areolar and fat tissue. In Series I and II, which we shall discuss together, rabbit cartilage was transplanted to guinea-pig. The effects of the heterotransplantation were followed in a continuous order from the eighth to the twenty-fourth day after the operations. Throughout there is some cartilage and perichondrium preserved up to the last period at which we examined the pieces. The amount of preserved tissue varies in different pieces. On the whole, in those parts in which the tissue shows signs of degeneration, either slight or more marked chromatolysis of nuclei or shrinking of cells and nuclei are observed, but in various other places cells and nuclei are apparently perfectly preserved up to the last. Often the more centrally situated parts suffer most, but not infrequently the peripheral cells are more injured than the neighboring ones. In certain specimens we had the impression that the interstitial cartilage substance takes up fluid from the surrounding tissue and shows signs of swelling, a condition which would be analogous to the swelling of corneal tissue, observed under certain circumstances. This effect however needs further investigation. As to the fat tissue, it soon becomes to a large extent replaced by host connective tissue and infiltrated by lymphocytes and polymorphonuclear leucocytes; but some fat tissue may possibly remain preserved. In some cases there exists a correspondence between the necrosis of cartilage and surrounding fat tissue. While thus at the end of the period of observation chosen by us the heterotransplanted cartilage is at least in places preserved, there is nowhere any attempt at regeneration noticeable on the part of the perichondrium, and in this respect the heterotransplant differs definitely from the homoiotransplant, where regenerative processes are a general occurrence. We must therefore assume that the hetero-toxins cause invariably an injury of the transplant, even in places where the structure of the tissue is apparently normal.

In Series III in which we transplanted rabbit cartilage to the rat, conditions are on the whole similar to those found in Series I and II. Cartilage and fat tissue are partly well preserved, partly necrotic. Even as late as twenty and twenty-four days after operation, there are usually parts of the transplanted tissue apparently alive. On the whole, the preservation of cartilage, as well as fat tissue, is perhaps a little better in the rat than in the guinea-pig. As far as the fat tissue is concerned this may perhaps be due to the fact that in the rat, as we shall see later, the host tissue usually invades the transplant less actively than in the guinea-pig. It may also be that the place of transplantation has a slight influence on the fate of the transplant. In the rat we generally used dorsal and in the guinea-pig ventral pockets. But in several cases we made use of ventral pockets also in the rat without much difference in the results, except perhaps in one instance. However, in the rat there is found just as complete an absence of regeneration as in the guinea-pig. We observed here, as well as in the rabbit cartilage transplanted into the guinea-pig, that when the cells disappear in the hyaline cartilage substance, they leave there in some cases a fine system of lines indicating the former outlines of the cells, so that almost a honeycomb appearance is produced. In a specimen obtained twenty-four days after transplantation, we noticed a softening and solution of the intercellular cartilage substance with the preservation of the cartilage cells. The latter are thus found lying in the periphery of the preserved cartilage, joined to each other by long fine processes. Thus the character of a structure somewhat resembling myxoid tissue was obtained.

In Series IV in which guinea-pig cartilage was transplanted to rats, specimens were examined from the first day to the twenty-eighth day. Again we obtained the same results, but it seems here that after the completion of the first week following the operation, the necroses of cartilage and fat tissue increase gradually and are especially marked from the eighteenth day on. However, as late as the twenty-eighth day some tissue is apparently still preserved. On the whole, the necrosis is more extensive in heterotransplantation than in homoiotransplantation; but there are always variable factors of a more or less accidental character which influence the amount of necrotic material found.

In Series V (transplantation of rat cartilage to guinea-pig), the transplant is relatively well preserved in the first four days; but

some cartilage is necrotic, especially in the center of the tissue, in case a thick piece of cartilage has been used. As in the preceding series, the amount of living transplant gradually decreases, but some preserved cartilage may be found up to the last day of examination.

If we compare the effect of heterotransplantation on the life and proliferation of various tissues we come to the following conclusion: While skin, thyroid and kidney tissue become usually entirely necrotic after heterotransplantation sometime between the seventh and fourteenth days after the operation, cartilage remains alive much longer; four weeks was the latest time at which we examined heterotransplanted cartilage tissue and found still some parts at least structurally preserved. Cartilage, being generally more resistant than the other tissues used previously, is much less readily destroyed under the influence of hetero-toxins; however, it would probably only be a question of time until the whole cartilage would be replaced by host tissue. On the whole, the amount of necrosis in cartilage seems to be greater after hetero- than after homoiotransplantation; and it increases gradually with the time elapsing after the operation. There is active, evidently, a hetero-toxin which has a direct injurious effect on the heterotransplant.

While epithelial tissues are more sensitive to hetero-toxins than cartilage, the former show a certain number of mitoses as the result of stimulation through transplantation, which are noticeable usually until a few days before death of the piece; no mitoses appear under these conditions in cartilage or perichondrium, apparently in accordance with the fact that cartilage and perichondrium have normally no marked tendency to proliferation by mitosis. However, the proliferation which we notice in the perichondrium after auto- and homoiotransplantation and which tends especially to replace necrotic cartilage, is lacking after heterotransplantation as the result of the injurious action of the hetero-toxins.

B. The Behavior of the Host Connective Tissue toward the Transplant

In homoiotransplantation of thyroid and various other organs and tissues, the connective tissue of the host actively invades the transplant and aids in its destruction. In heterotransplantation of kidney and thyroid, the connective tissue also surrounds the epithelial structures, forms hyaline tissue around them and through compres-

sion helps to destroy them. It may furthermore produce a capsule around the transplant as such. But on the whole the direct injury of the latter is so pronounced and the tissue is thus damaged to such an extent that the subsequent activity of the connective tissue plays only a relatively unimportant rôle in the process of destruction.

In heterotransplantation of cartilage the connective tissue of the host shows the following changes.

In Series I and II, rabbit cartilage was transplanted to the guinea-pig. Here the connective tissue is very active; from eight days on till the end, it invades fat tissue and replaces it gradually; soon it reaches the area surrounding the cartilage and forms a capsule about the latter. In various places, connective tissue cells and lymphocytes penetrate also into the cartilage, open the capsules of cartilage cells and fill them. At a period of twenty and twenty-four days following the transplantation, the connective tissue has invaded practically the whole transplant and replaced it, only a small part of the cartilage being left. Necrotic cartilage and probably also preserved cartilage have in this way been supplanted. At first, proliferation of the connective tissue begins at a distance from the cartilage; gradually it replaces more and more all of the tissue around the cartilage, presses on the latter and finally invades it. It is accompanied in its activities by lymphocytes and also by polymorphonuclear leucocytes. At early periods we may also see blood vessels extending through the transplant up to the cartilage.

In Series III, in which rabbit cartilage was transplanted into the rat, the activity of the connective tissue is on the whole less pronounced. The growth takes place mainly at a certain distance from the cartilage, but the connective tissue invades also fat tissue and in places supplants it. It penetrates the cartilage as well, especially where the latter is necrotic and where much hyaline intercellular substance has formed. In earlier periods especially (eight days after transplantation), blood vessels, with the connective tissue, almost reach the perichondrium. Conditions in the rat are then similar to those found in the guinea-pig, but on the whole the connective tissue activity is here somewhat less marked, except in a specimen examined fifteen days after transplantation into a ventral pocket, where the connective tissue activity resembles that observed in the guinea-pig.

In Series IV (guinea-pig cartilage into rat), the connective tissue

activity is distinct as early as three days after transplantation. At first, connective tissue with capillaries grows in the direction toward the cartilage; at later periods than the first week the blood vessels are not prominent. The connective tissue replaces gradually a great part of the fat tissue and penetrates also into the cartilage; but whether it penetrates preserved, as well as dead, cartilage is uncertain. As in homoiotransplantation, the cells move into the cartilage in the direction of the fibrils. The intensity of this connective tissue growth varies in different cases; in certain transplants only fibrous septa and nodules in the fat tissue are produced; in other cases the connective tissue replaces smaller or larger parts of the fat tissue.

In Series V (rat cartilage into guinea-pig), the connective tissue proliferation is noticeable two days after transplantation. The growth begins at first in the periphery of the transplant, but as early as ten days after the operation great parts of the fat tissue are replaced by fibrous tissue. Connective tissue begins also to invade the dead and probably also the preserved cartilage, but in various cases certain parts of the transplanted tissue may not yet be replaced by connective tissue as late as twenty-five days after the operation.

C. The Behavior of Polymorphonuclear Leucocytes and Lymphocytes toward the Transplant

Series I and II, rabbit cartilage into guinea-pig. Throughout the time of observation, from five to twenty days after operation, polymorphonuclear leucocytes are present, occurring first in the transplanted fat tissue, where they collect in large numbers, more particularly in places which are necrotic. Soon they begin to penetrate also a little way into the cartilage and are seen here also mainly in necrotic areas. They are especially successful in the invasion of cellular cartilage, but find it more difficult to advance into the hyaline cartilage substance. Cartilage cells may be in part digested by them. They are also seen in the fibrous capsule around the transplant and in the fibrous tissue around the cartilage. In different transplants and at various points of the same transplant, the number of leucocytes varies considerably. They may be rare in one place and in another may produce abscess-like formations. In the rat, these cells are less numerous than in the guinea-pig in which they remain active throughout the whole period of observation. Lymphocytes were likewise visible as long as observations were made, from eight to

twenty-four days after transplantation. They appear first in the periphery of the transplant, but soon move through the fat tissue, surround the cartilage and often penetrate the perichondrium and peripheral cartilage. As early as eight days after transplantation they may be found around the cartilage. Lymphocytes, together with some polymorphonuclear leucocytes and connective tissue cells, may fill the spaces which are surrounded by the hyaline capsules. In general, lymphocytes accompany the connective tissue and collect in large numbers in areas of fibrous thickening around the cartilage and in the fat tissue. We do not find that they move freely toward and into the heterotransplant in the manner in which they may invade homoio- or syngenesiotransplants. As a rule the infiltration becomes very dense in the course of the second week after transplantation, but again we note variations in the density of infiltration in the individual transplants and at different points of the same transplant. Occasionally giant and epithelioid cells are observed in the fat tissue; but usually they are not a very prominent feature in the heterotransplant.

Series III, rabbit cartilage into rat. The number of polymorphonuclear leucocytes is much less in the rat than in the guinea-pig. From the tenth day on, these cells may be very rare or lacking altogether. They are found especially in necrotic tissue. We observed them around fat needles which had formed in the transplanted fat tissue. They may also be mixed with the collections of lymphocytes. The infiltration of lymphocytes follows, on the whole, the same rules in the rat as in the guinea-pig, but in general it is less marked in the rat. These cells appear first in larger numbers in the peripheral parts of the transplant, but they soon reach the cartilage and may penetrate the peripheral cartilage, especially in places where it is necrotic. In the third week the infiltration may become dense, particularly at a distance from the cartilage, but quite decidedly also around the cartilage. As usual, lymphocytes accompany the connective tissue and in the latter often thick infiltrations may form. Some large mononuclear cells are occasionally mixed with the lymphocytes. At times giant cells are seen in the remnants of transplanted fat tissue.

Series IV, guinea-pig cartilage to rat. During the first three days polymorphonuclear leucocytes are found in the fat tissue and around the cartilage, either diffusely distributed or localized; sometimes

they occur in dense masses, especially where fat is necrotic. Some of these cells may enter perichondrium or cartilage; in the latter they degenerate, owing to the unfavorable conditions. In necrotic muscle tissue there are many polymorphonuclear leucocytes. Four days after the operation they begin to decrease in number and then to disappear; at five days some are seen degenerating in the cartilage. In the fat tissue a certain number of scattered cells of this kind are observed. In the following period up to the tenth day, and probably also later, there are a few polymorphonuclear leucocytes in the connective tissue around cartilage, but they are less numerous than in the guinea-pig. On the whole there are more of these cells in heterotransplantation than in homoiotransplantation. As to the lymphocytes, some mononuclear cells appear in the fat tissue during the first three days after tranpslantation. After three days a few lymphocytes can be seen in this tissue and in necrotic cartilage; but they appear in masses only at four days at the periphery of the transplant in the proliferating connective tissue. Also in the fat tissue and in the necrotic connective tissue some lymphocytes are visible. From this time on, many of these cells appear in the growing connective tissue; but there are, besides, numerous scattered lymphocytes in the areolar tissue and in the fat tissue. After seven days there may be a moderate or a considerable lymphocytic infiltration around the cartilage and in the fat tissue. Lymphocytes penetrate also a little into necrotic cartilage. On the whole the lymphocytic infiltration is densest at a distance from cartilage, in the proliferating connective tissue. There is a similar distribution of lymphocytes at nine days, although these cells occur also in the tissue somewhat nearer the cartilage; directly around cartilage there are fewer to be found than at a distance from the cartilage, in the growing connective tissue. In the fat tissue, lymphocytes are arranged especially around vessels and in the fibrous tissue traversing the fat tissue. At ten and eleven days there may be distinct lymphocytic infiltration in the connective tissue around cartilage. Dense lymphocytic infiltration is usually seen in the fibrous tissue; otherwise in the fat tissue and in the perichondrium only scattered lymphocytes are observed. Some lymphocytes may even invade living cartilage. At fourteen days we found the cartilage surrounded by a mantle of these cells several times thicker than the width of the cartilage. They penetrate also into the perichondrium and even more deeply into

the cartilage where it is necrotic. However, the density of the lymphocytic infiltration varies in different pieces. From now on until the end of the fourth week the conditions are similar; there may be moderate or dense infiltration around the cartilage and the lymphocytes may invade the peripheral cartilage in the direction of the fibrils. After twenty-eight days lymphocytes and connective tissue penetrate into the necrotic cartilage. There may be a dense lymphocytic infiltration around the cartilage, even in places where there is no marked connective tissue growth. On the whole this infiltration is more pronounced in heterotransplantation than in homoiotransplantation; but in general, in the former it accompanies the new formation of connective tissue, although at later periods there may be marked lymphocytic infiltration more or less independent of fibrous tissue proliferation.

Series V, rat cartilage to guinea-pig. Polymorphonuclear leucocytes are found as early as twenty hours after transplantation; during the first three days they are seen in the fat tissue, especially where it is necrotic, and around the cartilage. They penetrate into the dead cartilage, but also into living tissue between living perichondrium and cartilage; we find them situated particularly around blood vessels, but may see rather dense collections in various places. They are more numerous in these heterotransplants than in homoiotransplants. In the following period they diminish in number less markedly in the guinea-pig than in the rat. Thus at ten days, polymorphonuclear leucocytes migrate through necrotic fat tissue towards perichondrium and cartilage. They may be arranged diffusely or in localized collections. In the fat tissue and around cartilage some of them may disintegrate. The same condition is found in later periods up to the twenty-fifth day, but more and more lymphocytes begin to prevail, especially where a new formation of connective tissue has taken place. There are, however, polymorphonuclear leucocytes mixed usually with the lymphocytes and particularly in areas of fat tissue, the former cells may be seen almost exclusively; these may themselves become necrotic. The lymphocytes appear somewhat later than the leucocytes. Two days after transplantation we find a collection of lymphocytes and some larger mononuclear cells in the periphery of the transplant. Somewhat later they occur in considerable number, at a distance from the cartilage where the connective tissue is proliferating, and from here they penetrate into the

fat tissue. Thus in the second week there are found more or less dense collections of lymphocytes in the growing connective tissue. These cells now penetrate, on the one hand, somewhat outwardly into the adjoining muscle tissue of the host and, on the other hand, in the direction of and a little distance into the dead or living cartilage. There may develop considerable accumulations of lymphocytes in the fat tissue and also around the cartilage, especially where connective tissue is growing. At fifteen days we see in the fibrous tissue around the cartilage lymph vessels filled with lymphocytes and a marked lymphocytic infiltration extends to the cartilage. From the eighteenth to the twenty-fifth day we may find a similar dense or moderate infiltration in the connective tissue around the cartilage and in the fat tissue and at this time lymphocytes are therefore often very prominent.

Comparison between Homoiotransplantation, Transplantation into Different Varieties, and Heterotransplantation in the Rat

It is of interest to compare the reactions, in these three types of transplantation, of a relatively resistant tissue like cartilage. At twenty or twenty-one days after transplantation, there is usually, in ordinary homoiotransplantation, much connective tissue growth and this tissue generally replaces considerable parts of the fat and areolar tissue. The lymphocytic reaction is noticeable; but both connective tissue and lymphocytic reactions vary in intensity in different pieces. There may be some necrosis in the transplant, especially in the central parts, but the latter may show degenerative changes even in the normal, not transplanted xiphoid cartilage; areas of solution also occur. In other places as well, areas of cartilage may be necrotic. Bone marrow is replaced by fibrillar connective tissue. Regenerative new formation of cartilage on the part of the perichondrium is, in many cases, quite marked around necrotic areas in the transplanted cartilage. If we compare with the results of these typical homoiotransplants, those of the transplantation of cartilage into rats with different color pattern, we find, in general, that the amount of necrosis of transplanted tissue is greater in the latter case. Perichondrial new formation of cartilage is here very rudimentary or absent. Transplanted bone is necrotic, while in homoiotransplantation it may be partly preserved. The new formation of connective tissue

and replacement of fat tissue by fibrous tissue are more pronounced than in homoiotransplantation and the lymphocytic reaction is likewise more marked.

In heterotransplantation, the direct injury of the transplanted cartilage is usually greater than in either of the other two kinds of transplantation. There are as a rule more degenerative changes and areas of necrosis in the cartilage and perichondrium. Not rarely we find that especially the peripheral parts of the transplant are injured to a greater extent than the central parts. While in homoiotransplanted cartilage there may be areas of solution in the cartilage, these are usually lacking in the heterotransplants, although we found in the periphery of one of the latter transplants hyaline cartilage substance dissolved and the preserved cartilage cells forming a myxoid-like tissue. Perichondrial new formation of cartilage as well as the formation of nuclear chains, which are observed in homoiotransplanted striated muscle, are entirely lacking in heterotransplantation. On the other hand, here the formation of connective and especially of fibrous tissue is most pronounced, and the fat tissue in particular is in the course of time more and more replaced by fibrous tissue produced by the host. Lymphocytic infiltration is also very prominent in heterotransplantation. One characteristic feature here is that this infiltration is usually associated with connective tissue proliferation; this may be due to the fact that the latter process is so very marked in heterotransplantation. Furthermore connective tissue and lymphocytes usually penetrate together into injured parts of the cartilage; however, lymphocytic infiltration may occasionally be pronounced in places, when connective tissue new formation is apparently not very prominent. On the whole, it may be said that lymphocytic infiltration is more intense in hetero- than in homoiotransplantation or in transplantation into different varieties. In the latter two types, polymorphonuclear leucocytes are lacking at this period, while they are usually found in the first type, although in smaller number in the rat than in the guinea-pig.

Between five and eighteen days we find in principle the same differences between homoio- and heterotransplantation. The lymphocytic infiltration in the latter case is, in the earlier periods, especially marked at some distance from the cartilage and in the more peripheral parts of the transplants. At seven days the transplants into different varieties are already in some respects distinct

from homoio- as well as from heterotransplants. As compared with homoiotransplants, we find in the case of transplantation into strains with different color pattern (variety transplants) a greater amount of necrosis and an absence of perichondrial regeneration; there is also a beginning connective tissue activity and a moderate lymphocytic infiltration, the latter being more marked at this period than in the average case of homoiotransplantation. In heterotransplants on the other hand, the necrosis of the transplanted tissue, as well as the connective tissue proliferation and probably also the lymphocytic infiltration, is still more marked. There are also more polymorphonuclear leucocytes present in the heterotransplant, the greatest intensity of invasion by these cells being especially observed in the first few days; some leucocytes, however, may be seen even in the homoiotransplant in the first three days after operation.

We may then conclude that in (1) homoiotransplantation, (2) transplantation to different varieties and (3) heterotransplantation there is a gradation of reactions of the host and a corresponding gradation of effects on the transplant which become the more severe the more distant the genetic relation between host and transplant.

The Reactions of the Host against Heterotransplanted Tissues in General

We have studied the effect of heterotransplantation on various tissues, namely skin, thyroid, kidney and cartilage. In all these cases we were able to compare heterotransplantation with auto- and homoiotransplantation. Furthermore, we carried out experiments on homoiotransplantation of blood clots and we compared it, in some additional experiments, with heterotransplantation of blood clots. Thus we can, on the basis of these observations, arrive at some more general conclusions concerning (1) the effect of heterotoxins on tissues and (2) the reactions of the host cells against heterotransplants. As far as heterotransplantation of blood clots is concerned, the number of our experiments is as yet relatively small and the conclusions must therefore at present be considered as provisional.

1. The effect of hetero-toxins on tissues. If we transplant living tissue into a different species, there is noticeable a direct injurious effect of the body fluids on the transplant. Under these conditions, some constituents of the body fluids act as hetero-toxins. This is

true for all tissues so far investigated by us and it may even hold in the case of blood clot, where hemolysis seems to be more marked after hetero- than after homoiotransplantation. However, the power of resistance of various tissues varies. Skin, thyroid and kidney die between the seventh and fourteenth day after heterotransplantation; bone marrow and striated muscle tissue die rapidly after heterotransplantation; fat tissue also perishes, but in this case it is difficult to determine the exact time of complete necrosis. Cartilage, on the other hand, may remain preserved in part for four weeks or perhaps even somewhat longer; at least there are parts of this tissue which behave toward ordinary stains like normal tissue. Even in the case of cartilage, however, the amount of necrosis is greater after heterotransplantation than after homoiotransplantation or after transplantation into different varieties. It is known that certain transplantable tumors can remain alive and grow for an even longer period than cartilage, if the transplantation has been made into an animal of a different but not very distant species. However, after heterotransplantation, even those parts of tissues which are morphologically preserved are injured more intensely than after homoiotransplantation. This may be concluded from the fact that regenerative phenomena are diminished or lacking altogether after heterotransplantation. Tissues, such as skin, thyroid and certain tubules of the kidney, which naturally are more susceptible to growth stimuli, or which respond more readily to growth stimuli with mitoses, may still show mitoses after heterotransplantation, although these are less numerous than under more favorable conditions. Usually they cease a few days before the tissue becomes entirely necrotic; however, some mitoses may still occur near the time of death. In the case of perichondrium or peripheral cartilage, on the other hand, in which under favorable conditions regeneration takes place in response to the stimulation exerted by necrotic cartilage tissue and in which mitoses are rare and the tendency to cell proliferation is apparently less pronounced than in the case of certain other tissues, no trace of regeneration has been observed by us after heterotransplantation. Also, in heterotransplanted striated muscle, the formation of nuclear chains, which may be seen after auto- and homoiotransplantation, is completely lacking. Homoio-toxins, on the other hand, affect directly only very sensitive tissues, like the myxoid connective tissue of the uterus, unstriated muscle tissue and

probably bone marrow, while some other tissues are rather resistant to homoio-toxins. But all these tissues are severely affected by hetero-toxins. We may then conclude that hetero-toxins produce much more intensely a direct injurious effect on transplanted tissues than do homoio- or syngenesio-toxins. This effect is noticeable in a diminution in regenerative processes as well as in a relatively rapid destruction of tissue; however, certain differences exist in this respect between the various tissues and the naturally more resistant tissues have also a greater power of resistance to the action of hetero-toxins.

2. If we now consider the reactions of the host tissues against the transplant, we find again essential accordance in the different kinds of heterotransplantations, but certain variations occur due to conditions of secondary importance. In all cases the connective tissue proliferates and has a tendency to form fibrous tissue around and between the structures of the transplant, pressing upon the latter and thus injuring it. It invades and largely replaces transplanted fat tissue. Cartilage also is invaded, especially necrotic areas; but apparently certain parts at least partially preserved are invaded as well. In the case of cartilage, furthermore, we found that fibrous tissue forms in larger quantity around the transplants than around transplants of thyroid or kidney. This is probably due to the fact that cartilage is less rapidly destroyed than the two latter tissues and it can thus exert an effect upon the host which extends over a longer period of time; however, we saw the same reaction also during the second week in a few cases of thyroid and kidney transplantations.

A further reaction of host to transplant consists in the collection of large masses of lymphocytes in the connective tissue at some distance from the transplanted piece. This as well as the connective tissue proliferation we notice especially when cartilage and fat are used, although as stated, we find it indicated also in the case of the other tissues. Having infiltrated densely the area around the transplant, especially during the second week, the lymphocytes then invade it together with the connective tissue. Cartilage, it will be remembered, resists to some extent the action of both of these agencies, at least for some time, though very considerable masses of lymphocytes may collect around the transplant. In the case of skin, thyroid and kidney heterotransplants, the invasion by lymphocytes is much less pronounced than when homoiotransplants are made;

but even here lymphocytes may during the second week begin to collect in the periphery in large masses. The reasons for a less active lymphocyte invasion of such hetero-tissues as the tubules of the kidney and the acini of the thyroid are: (1) the earlier death of heterotransplants as compared with homoiotransplants; during the second week the former have to a large extent been destroyed; (2) the diminished metabolism generally of heterotransplants; and (3) their imperfect vascularization, lymphocyte infiltration taking place largely by way of the lymph vessels invading the transplant.

As far as we can judge, the heterotransplanted blood clot behaves in a manner similar to the other heterotransplants, especially cartilage and fat: it is surrounded and gradually replaced by large masses of fibrous tissue and lymphocytes. It seems that relatively inert foreign bodies, such as agar and serum coagulated through the action of heat, become organized without calling forth a similar, very marked proliferation of connective tissue and a massing together of lymphocytes. However, in the case of connective tissue, its activity as far as the heterotransplant is concerned consists in all probability largely in a migration of fibroblasts toward and into the transplant rather than in their mitotic proliferation.

We must, therefore, conclude that specific toxic substances, the hetero-toxins, are given off by these transplants which cause a reaction on the part of the connective tissue and lymphocytes of the host. Furthermore, if we consider that hetero-blood clots and, apparently, also the dying or necrotic tissue may call forth these reactions, it becomes probable that the hetero-toxins do not depend for their activation on the characteristic metabolism of living tissue, as it seems homoio-toxins do. This conclusion would be in harmony with the fact that while immunization against homoio-tumors can be made only with living tissue, we can accomplish such an immunization against heterotransplants also with lifeless material. There is an additional difference between homoio- and hetero-toxins: the latter attract the polymorphonuclear leucocytes much more markedly than the former. After homoiotransplantation we notice only a few polymorphonuclear leucocytes in the first few days after transplantation, while after heterotransplantation they are usually more numerous and they persist much longer. To some extent these reactions of the host against heterotransplants are also influenced by the specimens into which they are transplanted. Thus

in the guinea-pig, the activity on the part of the leucocytes and probably also on the part of the lymphocytes is more pronounced than in the rat. Reciprocal heterotransplantations, reversal of host and donor, do not necessarily give the same results, as we found in our earlier heterotransplantations. The reaction depends upon the activity of the host, which is not affected equally by all kinds of differences between host and transplant, but by differences of a special kind.

Summary

1. The direct injurious action of the body fluids of the host on cartilage and fat tissue which have been transplanted into a strange species is greater than the corresponding effect following homoiotransplantation. This direct injury has been observed by us in various tissues which we examined after heterotransplantation. However, cartilage differs from other tissues through its greater resistance to injuries in general and to the action of hetero-toxins in particular. Heterotransplanted cartilage may remain in part preserved for at least four weeks; but even the apparently intact parts, as far as structure is concerned, are injured inasmuch as no regenerative growth of perichondrium is observed.

2. The reactions of the host against the heterotransplanted cartilage and fat tissue are pronounced. A very marked invasion of the fat tissue by connective tissue takes place; the latter then becomes fibrous and replaces the fat tissue to a large extent. The cartilage is enveloped by fibrous tissue and partly invaded, especially in places where it is injured. Large masses of lymphocytes gradually collect around the cartilage, the infiltration beginning at a distance from the cartilage but gradually affecting the greater part of the transplant. This infiltration is generally associated with the connective tissue invasion of the piece, but occasionally it may occur in certain places to some extent independently of the connective tissue. After heterotransplantation, polymorphonuclear leucocytes collect in large numbers in and around the transplant and in the connective tissue, invading the transplant in larger numbers than after homoiotransplantation. These cells also persist much longer around heterotransplants than around homoiotransplants, where they are usually found only during the first three days following transplantation.

3. Some quantitative variations exist in different species in the

intensity of the reaction against heterotransplants; but in principle the reaction is the same in all species examined.

4. Some differences also exist in the behavior of the various tissues after heterotransplantation; but these are due mainly to differences in the resistance of the tissues, their tendency to mitotic proliferation and the duration of their survival. In principle the reactions are the same in the case of all heterotransplanted tissues tested so far.

5. There are some indications that while in the case of homoiotransplants only actively metabolizing tissue elicits the reaction on the part of the host tissue, in the case of heterotransplants dying or dead tissue also may call forth a reaction.

6. In comparing the results of transplantation of tissues into different, not related, individuals, into different varieties and into different species we find a gradation in the directly injurious effects on the tissues as far as their preservation and power of regeneration are concerned, as well as a gradation in the intensity and character of the reaction of the host against the transplants. Connective tissue and polymorphonuclear leucocytes react most strongly against heterotransplants, while blood and lymph vessels grow least into heterotransplants. Lymphocytes surround and invade transplants into different varieties more actively than homoiotransplants; they are very active around heterotransplants, provided that the destruction of the latter does not proceed with such rapidity that the attracting substances given off by the heterotransplants are correspondingly diminished in quantity.

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A CASE OF CARCINOMA OF THE COLON ASSOCIATED WITH SCHISTOSOMIASIS (BILHARZIOSIS) IN A YOUNG WOMAN *

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It is well known, especially from observations in Egypt by Ferguson,[1] that carcinoma of the bladder frequently supervenes upon endemic hematuria, a disease caused by the invasion of the bladder by the eggs of the digenetic trematode, *Schistosomum haematobium*. In view of this, there is a general impression that schistosomiasis (bilharziosis), may be an inciting factor of carcinoma. Schistosomiasis has also been etiologically connected with certain cases of primary carcinoma of the liver by Yamagiwa [2] (*Sch. Japonicum*), by Mouchet and Fronville,[3] and especially by Pirie.[4] Intestinal schistosomiasis, although it may give rise to extensive circumscribed thickenings of the mucosa of the colon in the form of polyps or papillomas (Sinderson and Mills,[5] Dolbey and Fahmy,[6] Martinez[7]), shows no special tendency to be followed by carcinoma, as far as can be seen from the literature. Therefore, the case of associated schistosomiasis and carcinoma to be reported below, having occurred in an individual who was far below the usual cancer age, seems sufficiently striking to be of interest in this connection.

CASE REPORT

Maria M. (16398), a Porto Rican native, 18 years old, single and a cigarette maker by occupation, was admitted to the Presbyterian Hospital, San Juan, Porto Rico, Jan. 4, 1926, complaining chiefly of abdominal pain and distension. She stated that these symptoms first appeared two months ago and that they have gradually become very severe. During the last month she has also vomited almost every day and she has had no movement of the bowels for fifteen days. The family history of the patient is irrelevant. From her past history it appears that she had had measles and smallpox in child-

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hood; she also claimed to have had hookworm disease two years ago.

On examination the patient appeared acutely ill, her temperature was 99.2 F and the pulse rate 120 per minute. Nothing remarkable was found anywhere except in the abdomen. The latter was found considerably distended, somewhat rigid and tender throughout. Suspecting intestinal obstruction, the patient was operated upon immediately (January 4). On opening the abdomen (Dr. Galbreath), much fluid escaped and a mass was found involving the splenic flexure of the colon, apparently producing complete obstruction. A loop of ileum was adherent to the mass. The large intestine as well as the ileum was considerably distended down to the mass. Below the latter the colon was collapsed. The ileum was also discolored and its serosa as well as that over the mesentery was covered with plastic exudate. Because of the poor condition of the patient no attempt was made to resect the colon and relief from the obstruction was attempted by means of colostomy.

The patient returned from the operating room in a condition of profound shock and died at 6 P.M. A necropsy was not obtained, but the wound was reopened postmortem and the entire mass was resected from the colon.

Pathologic Findings. Grossly, the resected mass represents a funnel-shaped portion of the colon, measuring 12 cm. in length. About two-thirds of its length is occupied by a tumor mass which almost completely fills the lumen, allowing only a thin probe to pass with difficulty. In that region the wall is hard and retracted, showing on section almost complete replacement of the wall by a grayish tumor mass irregularly studded with many gelatinous areas. The intestinal mucosa, both above and below the tumor mass, is reddened and thickened. Several lymph nodes of the mesocolon are enlarged and indurated — obviously carcinomatous.

Microscopically, the tumor shows the picture of a colloid (gelatinous) adenocarcinoma in its central portion where the intestinal wall is almost entirely substituted by tumor elements and massive accumulations of mucus. In the peripheral parts it appears more like a diffusely infiltrating carcinoma, consisting of clear spherical cells distended with mucus and of various sizes but mostly large. Their nuclei are distorted and pushed to the periphery (signet-ring cells). The stroma of the tumor in these regions is mostly poorly

developed, but in some places it is quite abundant, giving the character of a scirrhus. The lymph node metastases show everywhere the picture of a diffusely infiltrating carcinoma composed almost entirely of signet-ring cells with very little stroma. This type of tumor and its variations being well known, it is not necessary to go into further detail of its structure.

The unusual microscopic feature in this tumor is the presence of an abundant number of lateral spined ova which are readily identifiable as the ova of *Schistosoma mansoni.* These are also found in the metastatic foci of the lymph-nodes, but they are especially numerous in the peripheral parts of the tumor and in the adjacent parts of the colon. Here in addition to eggs there are also found adult worms in many places in the veins of the submucosa. In a few of the ova the spines appear at the pole as in *Schistosoma haematobium* but after careful observation it seems probable that such eggs are the result of artificial displacement of the spines during the process of hardening and embedding. This is apparent from the distorted shape and displacement of the embryo within the eggs. At any rate, on dissolving pieces of tissue with caustic soda solution, many typical lateral spined ova are recovered and no structures identifiable as ova contain processes which can be interpreted as polar spines. The eggs are in various stages of development, many of them containing full grown miracidia and showing various stages of disintegration. In the peripheral parts of the tumor where the different layers of the intestine are still well preserved, the ova are especially numerous in the mucosa and submucosa, and scarce in the muscular coats. In the more central parts of the tumor they are present mostly in those regions where the stroma is well developed. They are infrequent where the mucus is most abundant.

The reaction of the tissue toward the parasites is the same in all parts of the tumor as well as in the surrounding uninvolved parts of the intestine, and conforms to the tissue changes generally described for schistosomiasis (Dew,[8] Martinez,[7] and others). The most common reaction found is in the form of a well circumscribed nodule of about the size of a miliary tubercle consisting only of young fibroblasts, in the center of which is enclosed the more or less degenerated ovum. Very frequently the fibroblastic areas are surrounded by a varyingly thick wall of small round cells. Occasionally such a nodule contains more than one ovum (two to five), and not infre-

quently one or more foreign body giant cells can be found encroaching upon the ovum within the tubercle ("bilharzial tubercle").

In many places also, the fibroblastic reaction is missing and the eggs seem engulfed entirely in a mass of small round cells. In other places the reaction seems more acute and consists chiefly of an infiltration with eosinophilic polymorphonuclear leucocytes for a considerable distance around the ovum. Such reactions are most frequent about the eggs in and around the mucosa, while in the muscularis, eggs are often encountered which hardly show any cellular reaction around them, being enveloped only in a thin fibrous tissue capsule. Another reactive change which must be ascribed to the invading parasites is the enormous thickening of the mucosa in the parts of the intestine immediately adjoining the tumor. This is due to hypertrophy of the glandular crypts as well as to a dense infiltration of the stratum proprium with neutrophilic as well as eosinophilic leucocytes, lymphocytes and plasma cells.

The destructive changes in the ova are most prevalent in the thickest parts of the tumor. Here the fibrous tubercles frequently show only the chitinous shells of the eggs or their fragmentary remains. Total or partial calcification of the eggs is comparatively uncommon, and while the best preserved ova are to be found mostly in acutely inflamed areas, lytic processes in many embryos of these areas are quite apparent.

Comment

The relation of the schistosomiasis to the carcinoma in this case is, of course, impossible to determine. From certain considerations it would appear that the two conditions might well be regarded as a coincidence. On the one hand, carcinoma in young individuals is probably of much more frequent occurrence than is usually assumed, as can be seen especially from a very recent study by Quensel.[9] Moreover, according to the statistics of the same author as well as those of others (Philipp,[10] Merkel,[11] and Staemmler[12]), the carcinoma of young individuals is most frequently an intestinal carcinoma. On the other hand, in localities where intestinal schistosomiasis occurs, carcinoma of the colon is, as mentioned above, not known to be especially common. Ferguson[1] who has pointed out the frequency of secondary carcinoma of the bladder to bilharzial lesions in Egypt, rather emphasizes the rarity of carcinoma of the

colon in the same country, although intestinal schistosomiasis is more liable to be accompanied by neoplastic changes. Nor does colon carcinoma appear to occur with especial frequency in Porto Rico where the index of intestinal schistosomiasis is, according to Martinez,[7] 2.16 per cent (Utuado) to 8.4 per cent (Mayaguex).

Yet the significance of metazoan animal parasites, especially helminths, as tumor-inciting agencies regardless of the nature of their action, cannot be doubted. This has been amply demonstrated experimentally (Fibiger,[13] Bullock and Curtis[14]). As to schistosomiasis, the notion that it is conducive to carcinoma in man seems well founded even judging only from the bladder carcinoma which follows endemic hematuria. In intestinal schistosomiasis, so-called precancerous lesions are common enough. Perhaps the failure of carcinoma to develop more frequently in the intestine than in the bladder is due to a more effective defensive power of the intestine against these particular parasites. Thus after considering the lessened carcinoma incidence for the age of the patient in our case together with the tumor-inciting properties of the parasites, the possibility that the latter were in some way responsible for the carcinoma, can hardly be disregarded.

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DESCRIPTION OF PLATES

Plate 100

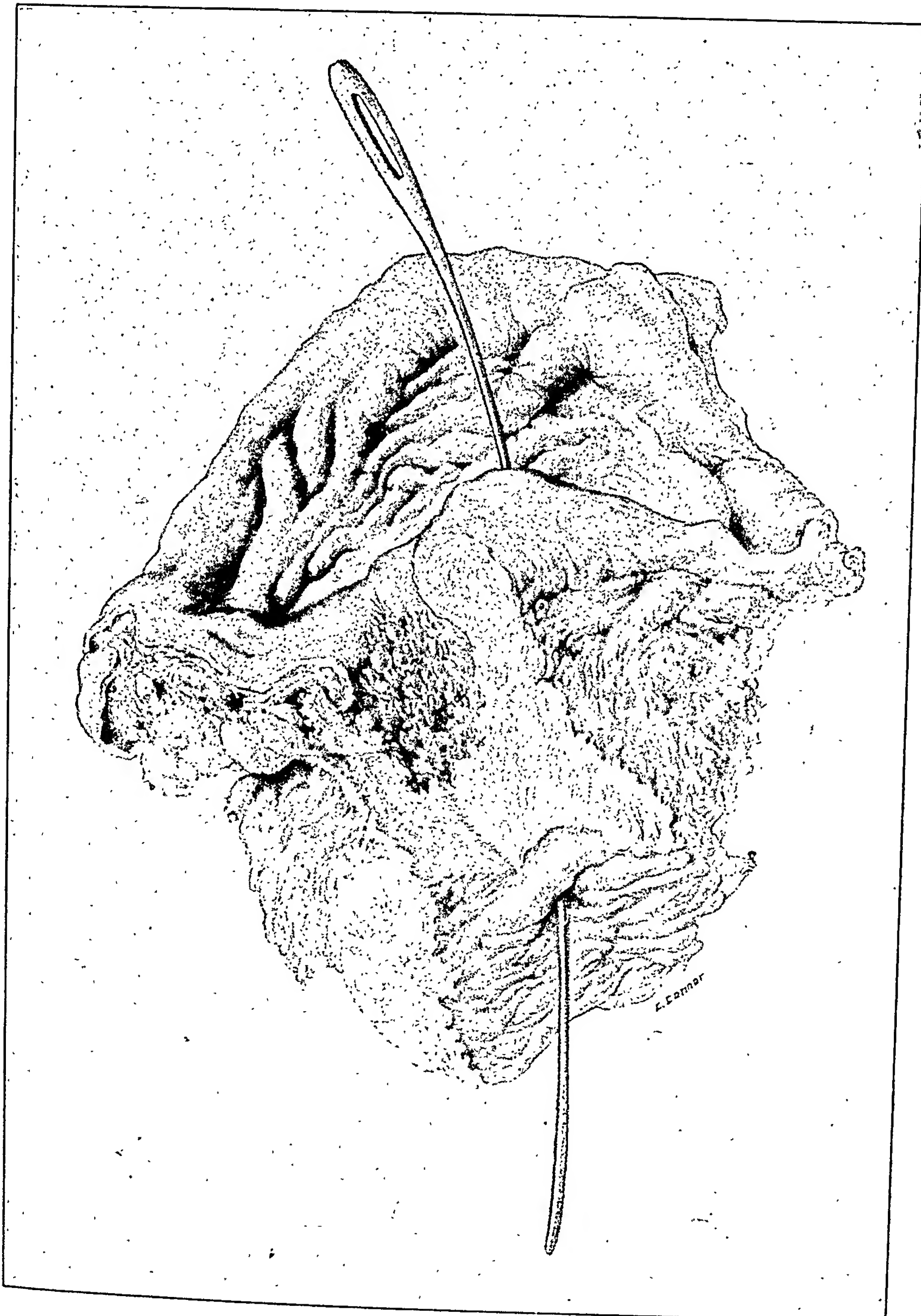
Fig. 1. Gross appearance of the resected colon, showing the tumor, with a probe in the stenosed lumen, and a tangential section of the wall.

Plate 101

Fig. 2. Microscopic appearance of the tumor in its central parts (adenocarcinoma).
Fig. 3. Cross-section of a vein containing a worm (male and female) in a scirrhous part of the tumor.

Plate 102

Fig. 4. Section of a submucosal vein containing three worms.
Fig. 5. Typical fibroid "tubercle" containing two ova and adjacent to it a nest of tumor cells surrounded by a wall of small cell infiltration.
Fig. 6. "Bilharzial tubercle" with a giant cell encroaching upon a lateral spined ovum.



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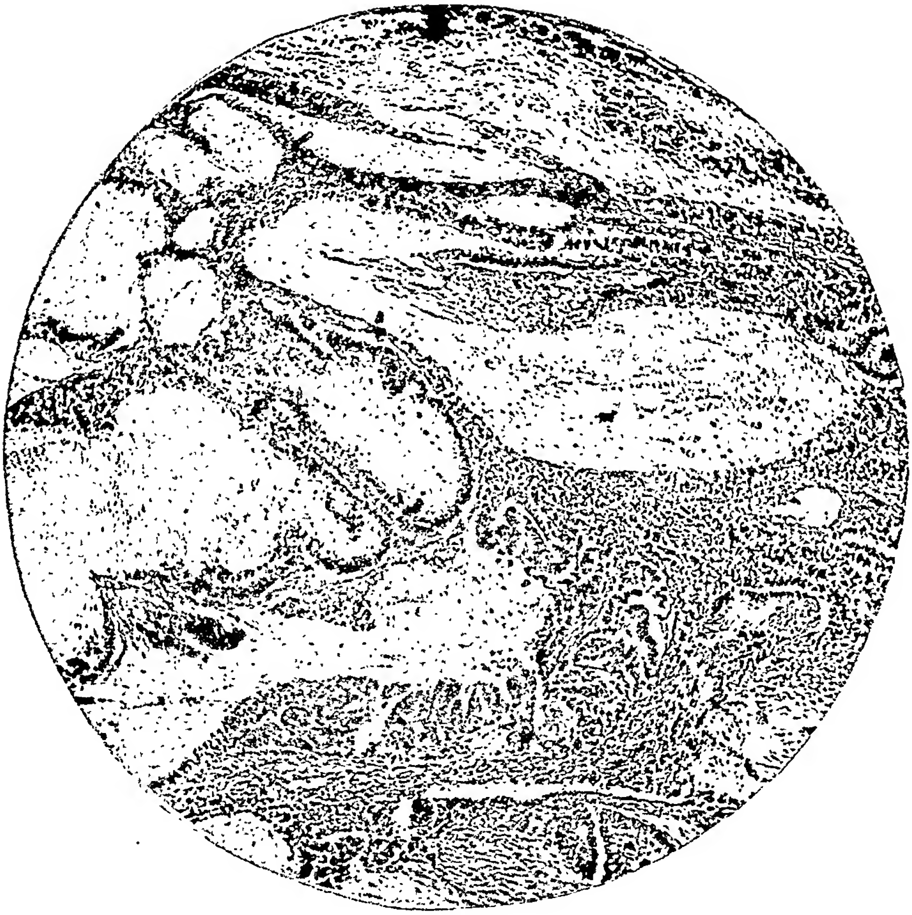
Roman and Burke

Carcinoma Associated with Schistosomiasis



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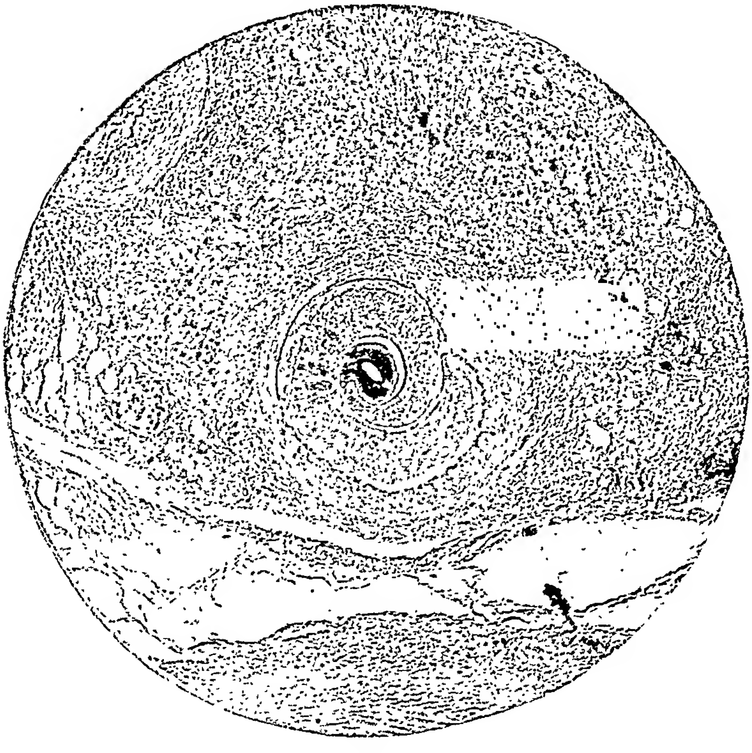

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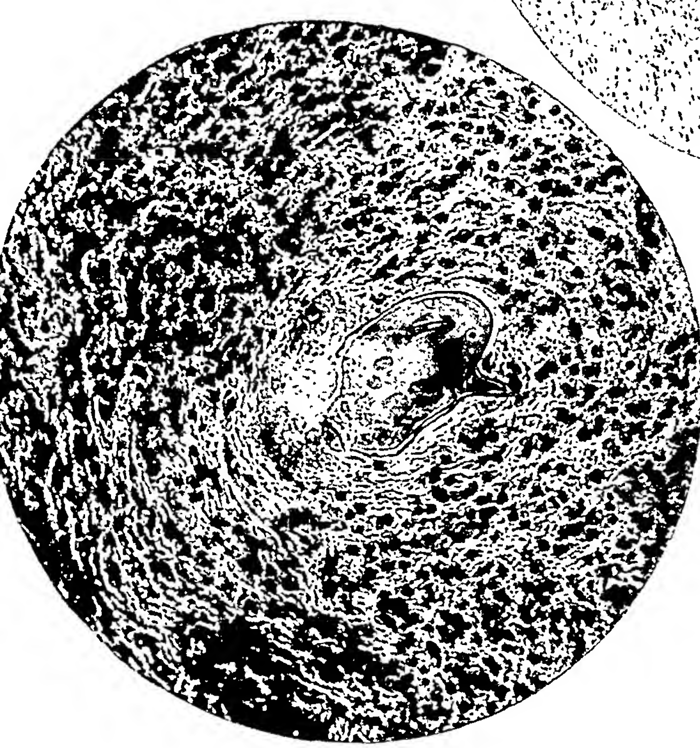

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Roman and Burke

Carcinoma Associated with Schistosomiasis

A STUDY OF THE TUMOR INCIDENCE IN THE LOWER ANIMALS *

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One of the greatest obstacles to a proper study of tumors in the lower animals is the lack of statistical data as to the total incidence in the various species of the different types of the disease. There is a great wealth of statistical evidence dealing with tumors in man but veterinary literature is woefully lacking in this regard. This is due largely to the fact that unlike the student of human medicine, the veterinary pathologist has no central agency of vital statistics to analyze and tabulate the results obtainable from death certificates. This information must come from animal clinics such as veterinary hospitals and institutions where accurate records are kept. The individual veterinary practitioner can contribute but little in this regard, although his carefully kept records for a number of years, if available, would have considerable value.

In reviewing the meager data on the frequency of tumors in the lower animals one is impressed with the fact that these formations are fairly common. The frequency of their occurrence is indicated by Kinsley's statement [1] that of 127 animals presented at the clinic of the Kansas City Veterinary College during one term, twelve were affected with neoplastic growths.

H. Martel, Chief of the Sanitary Veterinary Service, city of Paris, in an annual report [2] gives the results of statistical studies on the frequency of cancer among horses killed in abattoirs. Of 39,800 carcasses examined, mares constituted 2,000, geldings numbered 16,200, while 3,600 were stallions. Out of this number 184 were affected with cancer, distributed as follows: 86 in mares, 43 in geldings and 55 in stallions. The melanotic sarcomas were not counted since, Martel writes, "It is extremely rare to find white or gray horses entirely free from melanotic tumors." Most of the cases

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This is a condensed summary of a thesis presented to the Colorado Agricultural College in partial fulfillment of the requirements for a Master's degree.

were in subjects 15 years or older, while 118 horses of dark color were affected and 66 were found in whites and grays. Generalization was observed in 66 cases. As to locations affected, Martel found the 184 cases distributed as follows: kidney, 62; testicles, 50; mammae, 45; intestines, 9; bladder, 6; ovary, 2; lungs, 2; uterus, 1; sheath, 1; jaw, 1. The locations of five were not established. As to multiplicity, both testicles were involved in ten of the fifty cases, while out of the forty-five mammary tumors both glands were affected in six cases. The above figures are valuable but would be more so, if the various histologic types found were classified. From these figures it is evident that about one-half of 1 per cent showed malignant epithelial tumors and since cancer alone is mentioned, the total tumor incidence must have been much higher. The frequency of the disease in the kidneys, mammae and testicles is interesting for in these organs were located 157 out of the 184 cancers reported. Martel's figures also show a possible relationship between pigmentation and cancer since nearly two-thirds of the tumors occurred in the dark coated animals. This conclusion is perhaps not justified for no information is given as to the total number of dark and light horses respectively in the entire series studied (39,800).

Statistics from the Veterinary High Schools of Berlin, Dresden and Munich [3] show that 1.5 per cent of all horses, 4.5 per cent of all dogs, and nearly 20 per cent of all bovines presented for treatment were suffering from tumors.

Bemis [4] of Iowa reported that out of a clinic of 2,754 surgical cases, true tumors were observed in 27 per cent with about one-fifth of the cases malignant. Bemis' figures on incidence are considerably higher than others available.

In Dr. Kingman's clinic at the Colorado Agricultural College, in one series of 3,000 cases there were thirty-one cases in which true tumors were observed. This compares favorably with the German figures first quoted.

A surprisingly large number of fowls are victims of tumors and while reliable statistics dealing with this phase of tumor incidence are scarce, every poultry pathologist realizes that the percentage must be high; higher in fact than in mammals. In a recent article by Schneider [5] based on necropsies of all birds dying in a population of 11,000 individuals she reports the annual tumor rate for fowls between the ages of 6 and 18 months to be between 2 and 3 per cent.

Trotter [6] made an interesting report on malignant growths in the bovine from his study of 300 cattle suffering from malignant tumors. In the 300 individuals Trotter found 279 carcinomas and twenty-six sarcomas. (The difference in the total number of tumors in this instance was due to the fact that three of the animals had two primary growths while in one three primary tumors were found.) Only two of Trotter's cases were males (steers) while 298 were cows. Three cases were animals from 1 year to 3 years old while the rest, 297, were "aged." The locations of the tumors in Trotter's series were as follows: liver, 222; rumen, 25; thymus, 16; intestines, 10; lung, 8; ovaries, 5; bone, 1; skin, 1; eye, 4; vulva, 3; lymph glands, 3; kidney, 1; gall bladder, 1; uterus, 1; fascia, 1; salivary glands, 1; undetermined, 2.

The above statistics, while not in any sense exhaustive, should be sufficient to emphasize the fact that tumors of the lower animals are not uncommon but instead that they are rather frequently seen. The diagnosis of internal neoplasms in animals has not achieved the refinement practiced by the human physician and as a consequence the student of animal pathology is materially handicapped in having to depend largely upon an occasional necropsy for the chance discovery of internal tumors. Most animals dying on the farm are never examined postmortem and as a result comparatively few internal tumors are reported. In human medicine, on the other hand, internal neoplasms are usually diagnosed during life and an operation undertaken to rid the victim of the growth. To the number of tumors added to the human statistics from this source we have not a few added as the result of postmortem examinations to determine the cause of death in some obscure ailment. Then again, there is a greater interest on the part of the human physician and surgeon because of the attention neoplastic diseases command by their position in the annual mortality rates. Any disease that annually causes the death of between 90,000 and 100,000 of our population stimulates a certain earnestness on the part of those charged with the alleviation of human suffering and as a consequence we notice a coöperative effort toward the common goal that veterinarians usually fail to show.

While tumors of the lower animals do not occupy the same relative position of importance as tumors of man yet there is enough of practical worth to be learned to warrant a more sympathetic attitude on

the part of veterinarians in general toward this neglected field of pathology.

With the view of contributing something of value to the rather meager available information dealing with the histopathology, incidence, points of origin and location of the various groups of tumors as they occur in the lower animals, a series of studies was started some four years ago.

The work consisted of a thorough examination of all neoplastic material from the lower animals that could be obtained, the various specimens being studied grossly and microscopically, photomicrographs made and a diagnosis arrived at, using Mallory's nomenclature as far as possible. This report deals with a summary of the results up to this time.

In order to solicit the assistance of those most likely to encounter tumors in their daily routine, a form letter was addressed to thirty-five practicing veterinarians, most of whom were located in Colorado. Invitations to send in material were also sent to a half dozen or so of our graduates in the Government Meat Inspection Service and to a few veterinarians employed in clinical laboratories. In addition, the assistance of Drs. H. E. Kingman and James Farquharson of our college veterinary hospital was secured.

The response from the practitioners was somewhat disappointing. Ten or twelve have sent in one specimen, while four or five have supplied a goodly number. A large share of the material was obtained from packing house cases, although not a few were obtained through the kindness of other laboratories and our college veterinary hospital. Our own laboratory during the past two years has been a fruitful source, particularly of chicken tumors.

A summary of the source of the material received is as follows:

From practicing veterinarians	45
From packing house sources	38
From the College Veterinary Hospital	25
Other laboratories	33
Our laboratory	16
Total specimens received	157

This total included a number of specimens that were found not to be tumors when subjected to a microscopic examination and others that were not suited for study due to the lack of preservation and fixation. The total number of actual tumors studied was 132.

The 132 tumors studied were distributed among the various species as follows:

Kind of tumor	Mule	Horse	Dog	Bovine	Sheep	Swine	Fowl	Mouse	Rabbit	Total
Fibroma	3	5	.	5	.	.	.	.	.	13
Fibrosarcoma	3	3	1	2	.	.	.	.	.	9
Hypernephroma	.	..	.	4	.	.	.	.	.	4
Lipoma	.	1	1	..	.	.	.	.	.	2
Leiomyoma	.	..	.	2	.	.	1	.	.	3
Endothelioma	.	..	1	1	.	.	1	2	.	5
Mesothelioma	.	1	.	1	.	.	.	.	.	2
Myxoma	.	..	.	1	.	1	.	.	.	2
Myxofibroma	.	..	1	..	.	.	.	.	.	1
Melanoma	1	2	.	4	.	2	.	.	.	9
Melanosarcoma	.	2	1	..	.	1	.	.	.	4
Lymphocytoma	.	..	1	..	.	.	9	.	.	10
Lymphosarcoma	.	..	4	2	2	2	2	.	1	13
Lymphoma	.	..	.	1	.	.	.	.	.	1
Embryonal Carcinoma	.	..	1	..	.	.	.	.	.	1
Carcinoma	.	11	1	15	.	.	1	.	.	28
Adamantinoma	.	1	.	..	.	.	.	.	.	1
Adenocarcinoma	.	1	1	2	.	9	3	.	.	16
Adenoma	.	..	3	..	.	.	2	.	.	5
Papilloma	.	..	1	1	.	.	.	.	.	2
Cholesteatoma	.	1	.	..	.	.	.	.	.	1
Totals	7	28	17	41	2	15	19	2	1	132

The epithelial tumors occurred as follows:

Kind of tumor	Mule	Horse	Dog	Bovine	Sheep	Swine	Fowl	Mouse	Rabbit	Total
Carcinoma	.	11	1	15	.	.	1	.	.	28
Adenocarcinoma	.	1	1	2	.	9	3	.	.	16
Adamantinoma	.	1	.	..	.	.	.	.	.	1
Adenoma	.	..	3	..	.	.	2	.	.	5
Papilloma	.	..	1	1	.	.	.	.	.	2
Embryonal Carcinoma	.	..	1	..	.	.	.	.	.	1
Totals	.	13	7	18	.	9	6	.	.	53

Total number of epithelial tumors	53
Total number of non-epithelial tumors	89
Total number of all tumors	132
Percentage of epithelial tumors	40
Number of malignant epithelial tumors	46
Number of non-malignant epithelial tumors	7
Percentage of epithelial tumors that were malignant	86.8
Percentage of epithelial tumors that were not malignant	13.2
Percentage of malignant epithelial tumors to total number of tumors received	34.9

Malignant growths other than epithelial in nature are here classified.

Kind of tumor	Mule	Horse	Dog	Bovine	Sheep	Swine	Fowl	Mouse	Rabbit	Total
Fibrosarcoma........	3	3	1	2	.	.	.	.	.	9
Endothelioma........	.	.	1	1	.	.	1	2	.	5
Hypernephroma......	.	.	.	4	.	.	.	.	.	4
Mesothelioma........	.	1	.	1	.	.	.	.	.	2
Melanosarcoma......	.	2	1	.	.	1	.	.	.	4
Lymphosarcoma	.	.	4	2	2	2	2	.	1	13
Lymphocytoma......	.	.	1	.	.	.	9	.	.	10
Totals..........	3	6	8	10	2	3	12	2	1	47

Percentage of non-epithelial malignant tumors of total 35.6

Malignant tumors of all varieties are grouped according to the following table.

Kind of Tumor	Mule	Horse	Dog	Bovine	Sheep	Swine	Fowl	Mouse	Rabbit	Total
Carcinoma...........	.	11	1	15	.	.	1	.	.	28
Adenocarcinoma	.	1	1	2	.	9	3	.	.	16
Adamantinoma	.	1	.	..	.	.	.	.	.	1
Embryonal Carcinoma	.	..	1	..	.	.	.	.	.	1
Hypernephroma......	.	..	.	4	.	.	.	.	.	4
Fibrosarcoma........	3	3	1	2	.	.	.	.	.	9
Endothelioma........	.	..	1	1	.	.	1	2	.	5
Mesothelioma........	.	1	.	1	.	.	.	.	.	2
Melanosarcoma......	.	2	1	..	.	1	.	.	.	4
Lymphosarcoma	.	..	4	2	2	2	2	.	1	13
Lymphocytoma......	.	..	1	..	.	.	9	.	.	10
Totals..........	3	19	11	27	2	12	16	2	1	93

Percentage of total tumors that were malignant 70.4

Non-malignant growths of all varieties were found as follows:

Kind of tumor	Mule	Horse	Dog	Bovine	Sheep	Swine	Fowl	Mouse	Rabbit	Total
Fibroma............	3	5	.	5	.	.	.	.	.	13
Lipoma.............	.	1	1	..	.	.	.	.	.	2
Leiomyoma..........	.	.	.	2	.	.	1	.	.	3
Myxoma............	.	.	.	1	.	1	.	.	.	2
Myxofibroma........	.	.	1	..	.	.	.	.	.	1
Melanoma..........	1	2	.	4	.	2	.	.	.	9
Lymphoma..........	.	.	.	1	.	.	.	.	.	1
Adenoma...........	.	.	3	..	.	.	2	.	.	5
Papilloma..........	.	.	1	1	.	.	.	.	.	2
Cholesteatoma.......	.	1	.	..	.	.	.	.	.	1
Totals..........	4	9	6	14	.	3	3	.	.	39

Percentage of total tumors that were non-malignant 29.6

Points of Origin and Location. A summary of the locations occupied by the 132 tumors shows that the following were involved one or more times. Eye, esophagus, lip, brain, jaw, concha, glans penis, leg, pectoral region, shoulder, neck, mediastium, lung, uterus, spleen, vagina, axillary space, lymph nodes, gizzard, oviduct, kidney, sublumbar region, hip, testicle, spinal canal, pharynx, prepuce, adrenal body, walls of thoracic cavity, below ear, region of flank, abdominal wall, poll, anus, thoracic cavity, mesentery, intestines, heart and nasal cavity.

The organs most frequently affected were the following:

Kidney	9
Eye and its appendages	20
Lung	6
Liver	8
Spleen	5
Lymph nodes	10
Intestines	4

The kidneys were most often affected in hogs, the spleen and liver in chickens and the eye in cattle and horses (twelve and seven times respectively). The lymph nodes were involved most frequently in the dog.

In many of the cases two or more organs were affected by the same tumor such as the lung and the lymph nodes, or the liver, spleen and kidneys.

The incidence of the disease was apparently not influenced by sex, tumors occurring with equal frequency in the male and female. In a few instances there were more females than males represented; this predominance occurred in the case of old cows of which there are a larger number slaughtered than old males.

The influence of color was not suggested except in the case of melanoblastomas. All the horses affected with this tumor were gray, while the cattle and swine affected were red.

The age incidence could not be satisfactorily determined for all groups of tumors because of the failure of the clinician to record the age in every case. The groups in which sufficient number of ages were given enables me to offer the following data.

AVERAGE AGE OF ANIMALS AFFECTED WITH THE FOLLOWING TUMORS

Carcinoma:	Horse	10 years
	Bovine	6 years
	Canine	6 years
	Chicken	2 years (one case)
Lymphosarcoma:	Canine	6 years
	Sheep	2 years
	Bovine	7 years
Fibrosarcoma:	Horse	8 years
	Bovine	4 years
	Canine	3 years (one case)
Lymphocytoma:	Chicken	1 year
Adenocarcinoma:	Hog (kidney cases)	$2\frac{1}{2}$ years
	Bovine	4 years
	Avian	$2\frac{1}{2}$ years
	Horse	15 years (one case)

The numbers of different kinds of tumors that occurred in the various species follow:

Mule	3	Bovine	13
Horse	10	Sheep	1
Dog	12	Swine	5
		Fowl	7

The different species gave rise to the following number of tumors:

Bovine	41	Mule	7
Horse	28	Sheep	2
Fowl	19	Mouse	2
Dog	17	Rabbit	1
Swine	15		

DISCUSSION

From the available figures it is difficult to compare the frequency of tumors in the lower animals with the occurrence of neoplasms in man. That is, it is impossible, for instance, to say what per cent of the horse, dog or bovine population is affected with tumors at a certain age, while the information available does permit of such a statement as regards the human. The figures, as unsatisfactory as they are, do however suggest that there are probably but slight differences as regards the total incidence of tumors in the two classes (man and animals).

It is obvious that certain tumors appear with greater frequency than others and that there is quite a difference in the susceptibility of the various species towards neoplastic growth.

One cannot but be impressed by the large number of epithelial tumors encountered. In this series fifty-three cases or 40 per cent were of this variety and of these total malignancy was a feature in forty-six or 86.8 per cent. Malignancy seems to be the rule in the tumors of the lower animals as evidenced by the high percentage of malign growths in this study in which 70.4 per cent of all varieties possessed this feature.

The high incidence of malignant epithelial tumors is an interesting finding as is the fact that the epithelial malignancies practically equal the malignancies of all other kinds combined (forty-six epithelial malignant tumors and forty-seven non-epithelial malignant tumors).

A great many of the tumors such as the lipomas, mesotheliomas, lymphomas, myxomas and cholesteatomas were encountered but once or twice, which would suggest that these forms are comparatively rare in the lower animals. A few others such as chondroblastomas, osteoblastomas, rhabdomyoblastomas, neuroblastomas and glioblastomas, I was unable to add to my collection, and by failure to get material of these varieties from others, I am convinced that they must occur very infrequently.

The relative infrequency of neoplasms in sheep is another interesting point brought out by these figures. Only two of the 132 in the total were found in this animal. It is difficult to account for this apparent rarity on any other basis than a species insusceptibility. Of course, a great majority of sheep are slaughtered while lambs, and other than congenital neoplasms would have but little opportunity to develop during the short life of the animal. Again, however, a considerable number of old ewes come to postmortem examination annually, yet available data bear out the figures of my series and one must conclude that sheep are relatively immune to tumorous proliferations.

The mule is likewise peculiar in some respects. While the fibroblastomas commonly occur in this animal, other varieties must be rare. In the 132 cases reviewed, aside from the fibroblastomas, but one other tumor, a melanoma, was found. The racially close horse, on the other hand, was found to be particularly susceptible to the epithelial growths in addition to those affecting the mule. In comparison with all other species, the mule appears to be less subject to large numbers and varieties of tumors than any other domestic

animal, with the exception of the sheep. The horse, bovine and dog show the greatest susceptibility, with the bovine heading the list.

The most frequent tumors of the dog as they appeared in the above tabulations were the adenomas and the lymphosarcomas, while in the swine the familiar adenocarcinoma * of the kidney comes first. Aside from this one variety of epithelioblastoma, the hog is apparently infrequently affected with the epithelial tumors. In my cases no other types appeared.

The common fowl is likewise seldom affected with epithelial tumors and it is indeed rare that a true carcinoma is seen (but once in my series). By far the greatest number of chicken tumors belong to the lymphoblastomas. In my collection of nineteen neoplasms of the domestic fowl nine were lymphocytomas (leukemias) and two were lymphosarcomas. In no instance have I encountered neoplasms in the turkey, which seems unusual.

The bovine appears to be especially prone to tumors. In the cases previously listed forty-one tumors (about 31 per cent of the total) were secured from cattle and thirteen different types of tumors were represented in the total. In addition to the high tumor incidence in the bovine, this species seems susceptible to the greatest variety of new growths. The forty-one tumors listed as occurring in this animal consisted of thirteen different varieties. The dog is also interesting in this regard, the seventeen specimens from this species falling into twelve different groups, while the horse with twenty-eight tumors showed ten different kinds.

A large share of the epithelial malignant growths in the horse and bovine involved the eye and the appendages of that organ. Of the thirty-one epithelial growths affecting these two species, fifteen involved this organ with the disease apparently occurring most often in the bovine. The eye of the Hereford seemed to be particularly susceptible. The penis of the horse was also a common location occupied by carcinomas. Adenomas arise not uncommonly from the eye of the dog and the majority of the adenocarcinomas involved the kidneys of hogs (nine out of sixteen).

The distribution of the melanoblastomas is of some interest, being one in the mule, four in the horse, one in the dog, four in the bovine and two in the hog. Most workers have found this tumor far more

* I have used the term adenocarcinoma for that group of kidney tumors of swine commonly called adenosarcoma. A paper in support of this designation is in preparation.

frequent in the horse than in any other species. While these cases were scattered among the different species, I do not think that the figures should alter the accepted opinion in this regard. The scarcity of this tumor in the horse in my series is probably due to the comparatively few tumors in the total (132) and the fact that melanotic growths are so frequent in the horse that they were not considered of sufficient importance to be worthy of microscopic study. Again, melanomas are the easiest tumors to diagnose in the gross, and the curiosity which might have prompted the practitioner or meat inspector to send many other tumors to the laboratory for a diagnosis was absent in the case of these pigmented growths. The occurrence of this tumor in the other species suggests that perhaps the bovine, hog, mule and dog have more melanoblastomas than is ordinarily appreciated.

A tumor that is even more frequent than this report would indicate is the leukemic condition in chickens, more properly termed malignant lymphocytoma. In addition to those which appear in this work we have had a considerable number of these cases in our laboratory for diagnosis during the past few months. In fact this is without question the most frequent neoplasm with which chickens may be affected. Of 101 chickens passing through our laboratory during the past four months, eight, or 8 per cent, showed this condition.

It is not presumed that the figures offered in this report on the incidence of the various tumors in the different species are correct for the entire animal population represented. To the contrary, many of the percentages would probably be found decidedly erroneous if a sufficiently large number of specimens could be collected. They do, however, represent the approximate incidence in at least certain of the groups. This is more especially true with the epithelioblastoma and certain of the lymphoid tumors.

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PRIMARY CARCINOMA OF THE LIVER OF POSSIBLE MULTICENTRIC ORIGIN OCCURRING IN A CASE OF PORTAL CIRRHOSIS *

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In a recent paper we presented a clinico-pathologic study of five cases of primary carcinoma of the liver occurring at the Mayo Clinic. As is well known, this rare tumor occupies a somewhat unique position, not only on account of its habits of growth and spread, but also by reason of its almost constant association with cirrhosis. Particular attention was paid to this relationship, both in the cases we reported and in those collected from the more recent literature. The incidence of cirrhosis, as given by the earlier writers, Eggel, Goldzieher and von Bókay, and Yamagiwa, varies from 70 to 80 per cent, especially for the hepatoma or primary liver cell form, but later reports indicate an even higher percentage and that cirrhosis is the main predisposing factor in the disease. Thus the cases reported by Karsner and by Winternitz, and nineteen occurring since 1922, all showed cirrhosis. The case of Helvestine is the only one in which it was absent.

Concerning the multicentric or unicentric origin of carcinoma of the liver there is considerable divergence of opinion. Winternitz, Karsner and others hold that the disease is unicentric and that its spread in the liver is metastatic by way of the portal and hepatic veins. Van Heukelom and Travis have described transitional forms at various points in the neighborhood of carcinomatous nodules and have concluded from this that the growth is multicentric. It is indeed on the basis of the presence or absence of these transitional forms that practically all writers have based their conclusions. It seems, therefore, that the question will never be satisfactorily settled so long as it depends on the individual interpretation of various stages of the carcinoma cell, a matter of extreme difficulty and varied interpretation under the best conditions. While our

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studies on this point led to nothing definite which might aid in the solution of the problem, our inability to find any such transitional forms forced us to agree with Winternitz and Karsner that in all probability the tumor was unicentric in origin. All of our cases were advanced, so that clear evidence of the earliest stages was difficult to obtain. However, the recent occurrence of a remarkably early case of primary carcinoma of the liver has caused us not only to reverse our previously expressed opinion but to bring forward evidence on a different basis, suggesting that the condition can be multicentric in origin as well as metastatic in spread.

Report of a Case

A woman, aged 58 years, was admitted to the clinic complaining of stomach trouble. For the previous twelve years she had suffered attacks of epigastric pain with nausea and vomiting followed by considerable abdominal soreness. For the previous year the attacks had become so severe as to require sedatives, and were accompanied by edema of the feet and progressive swelling of the abdomen.

The patient was moderately jaundiced and had edema of the legs and feet and marked swelling of the abdomen due to fluid. The liver and spleen were not palpable. Large external hemorrhoids were present. The hemoglobin was 77 per cent. Erythrocytes numbered 4,090,000, and leucocytes 7,800 per cu. mm. The phenoltetrachlorphthalein test of hepatic function showed dye retention 3. Roentgenograms revealed multiple small stones in the gall bladder. A diagnosis of cholelithiasis with secondary cirrhosis of the liver was made. On exploratory operation the diagnosis was confirmed and a Talma-Morrison operation was performed. Paracentesis was carried out twenty-one days after operation but the patient gradually became weaker and died thirteen days later.

Necropsy showed portal cirrhosis of the liver with early primary carcinoma, ascites, bilateral hydrothorax and anasarca, dilatation of the veins of Sappey, of Retzius and of those of the esophagus, cholecystitis and cholelithiasis with dilatation of the gall bladder and ducts (Grade 2) and arteriosclerosis (Grade 3).

The skin and sclerotics were markedly jaundiced. The abdominal cavity contained 1,500 cc. of fluid while the pleural cavities together contained about the same amount. The subdiaphragmatic, intestinal and inferior hemorrhoidal veins were all markedly dilated and tortuous. The spleen weighed 209 gm. and presented patchy hyaline thickenings of the capsule with considerable fibrosis of the pulp. The gall bladder had thickened, opaque, white fibrous walls and contained 50 cc. of greenish black bile together with multiple, small, dark, faceted stones.

The liver weighed 846 gm. without the gall bladder. Its surface was yellowish white with a thick, tough, fibrous capsule covering a surface roughened all over by more or less coalescent nodules, from 2 to 4 mm. in diameter, giving the organ a finely granular appearance. On section it offered considerable resistance to the knife and presented a cut surface which was markedly granular, corresponding to the external appearance. Each of the small, rounded, yellowish areas of

hepatic tissue measured from 2 to 4 mm. in diameter, and was surrounded by a band of dense, glistening, fibrous tissue. In addition to this typically cirrhotic state there were three well defined, soft, greenish white nodules averaging 2 cm. in diameter and surrounded by dense connective tissue. From the capsules of each, a fine network of fibrous tissue radiated to the center, dividing the contents into smaller granules and supporting the rather pasty friable mass which constituted the nodules. Two of them were situated at the extremity of the left lobe while the other was in the right lateral portion of the right lobe. Gross serial sections showed that they could all be included in two slabs (Figs. 1 and 2) and that no other similar areas existed in any situation. From the photographs it can be seen that one piece (Fig. 1) included both nodules in the left lobe (*a* and *b*), and part of the nodule in the right lobe (*c*). The other included the remainder of the nodule (*c*) (Fig. 2).

Pathologic Analysis

The surfaces of these slabs were then divided into definite numbered areas, and thin sections shaved from their whole extent for microscopic examination. Thus a series of large microscopic slides was prepared, each from a known area and representing the entire breadth of the liver in two planes. A careful search was made of all other organs for a possible primary focus. As none was found, the tumors were assumed to be primary in the liver rather than metastatic.

Microscopic Examination. Examination of the three nodules, *a*, *b* and *c*, shows them all to be of the same nature. Each is a partially encapsulated mass of cells quite irregular in arrangement and showing none of the characteristic radiate disposition of normal hepatic tissue. Nodule *a* consists of masses of polyhedral, triangular, round and oval cells varying greatly in size but very little larger than normal hepatic cells. For the most part, they have a definite trabecular arrangement forming anastomosing columns, with here and there many tubules which contain bile (Figs. 3 and 4). The cells are supported by a delicate stroma of connective tissue, more marked in some places than in others, although this is not a pronounced feature. Endothelium-lined spaces everywhere separate the cell columns; they are obviously vascular capillaries. The cells themselves are fairly easily distinguishable from the surrounding parenchyma of the liver, the nuclei being irregular, markedly hyperchromatic and basophilic, often multiple (Fig. 5), and exhibiting occasional mitotic figures (Figs. 6 and 7). The cytoplasm is more acidophilic than usual and is finely granular in appearance. Toward the center of the nodule degenerative changes are common, especially fatty vacuolation

of the cytoplasm, with nuclear pyknosis and karyolysis. Scattered throughout the capillaries are many polymorphonuclear and lymphocytic leucocytes.

Nodules *b* and *c* prove to be identical histologically with *a* except that fatty degeneration is much more prominent. The capsule of all three consists of dense fibrous tissue containing a few regenerating bile ducts and columns of hepatic cells. While a capsule is found around each nodule it does not in any way confine the tumorous tissue within its limits. Columns of malignant cells are found actively growing through and outside it, invading the parenchyma for a short distance on all sides (Fig. 8). It is evident that the capsules are condensations of the sclerotic tissue already present in the cirrhotic liver and that they are produced by the expansion of the carcinomatous tissue. A study of the tissue intervening between the nodules proves of the greatest interest. Scattered in the parenchyma between *a* and *b* are several masses of cells identical with those in the larger nodules. While some appear to be lying free among the hepatic cells, others are obviously within endothelium-lined spaces or vascular channels. It seems therefore that these are metastatic growths and represent the path of extension either from *b* to *a*, or *vice versa*. With the exception of this area and of that just outside the capsule of the nodules, a close and careful examination of all the other slides, particularly from the area between *b* and *c*, fails to reveal any sign of definitely malignant cells.

The cirrhotic areas themselves were next examined. An extremely severe chronic hepatitis is in progress with diffuse infiltration of the liver columns by masses of lymphocytes. These are for the most part collected in and around the dense fibrous tissue which surrounds the nodules of the liver parenchyma, although a more diffuse and intercellular type of infiltration is not uncommon. A general view of any one of these nodules shows it to consist of irregularly anastomosing columns of double liver cells, devoid of any normal or lobular arrangement with regard to the central vein. In no area can these central veins be distinguished, although endothelium-lined spaces in the surrounding fibrous tissue indicate that they have been engulfed by it in the constant destructive and constructive processes at work. Each nodule shows considerable fatty degeneration, bile stasis with bile thrombi and venous congestion.

It was to the individual cell, however, that more particular atten-

tion was directed. Where the hepatitis is more diffuse, regenerative and hyperplastic forms are evenly distributed throughout the nodules, along with the débris of cells in the process of dissolution. Generally speaking, this regeneration is more marked at the periphery than at the center, where atrophic forms are much more numerous. The chief distinguishing points in these newly formed cells are their size, shape, staining reactions and characteristic nuclei. They tend to be larger and more convex, with clear cut edges and more acidophilic cytoplasm than the normal cell. The normal nuclei are usually fairly uniform in size and are composed of a moderately basophilic lacework of chromatin, often containing a single nucleolus. In any field on the slides the eye is immediately attracted to these hyperplastic cells by reason of the larger and more irregular nuclei, and by their more basophilic and hyperchromatic tendencies. Although mitotic figures are infrequent, binucleate, trinucleate and multinucleate forms are relatively common. Between the single and binucleate cells, dumb-bell forms are occasionally noted, while much larger cells, containing as many as nine nuclei, are also found. These multinucleated forms occur in practically every regenerating nodule in the cirrhotic areas, yet none can be definitely classed as malignant.

Discussion

It is important, in the first place, to distinguish between the condition we have described and the so-called nodular hyperplasia or adenomatous regeneration of the liver. The term hepatoma, later appropriated by Yamagiwa for the liver cell type of carcinoma, was originally used by Sabourin for this condition. He considered it to be an intermediate stage between cirrhosis and carcinoma, a view held by many subsequent writers. According to Mallory it is found more frequently in those acute toxic types of cirrhosis in which degeneration and regeneration are so rapid that excessive new growth of liver cells results in the formation of greenish yellow, encapsulated nodules 1 cm. or more in diameter. Aschoff, Rolleston and Ribbert emphasize the difficulty in making a gross diagnosis between the two conditions, and Hansemann states that adenoma may pass into carcinoma with no definite line of demarcation. Ribbert, indeed, holds that the liver cell type of carcinoma almost invariably represents malignant change within these adenomatous masses.

Well marked examples are, however, distinguished microscopically by the degree of differentiation of the cells and by their non-invasive characteristics. While the cirrhosis in this case was of an extremely chronic type, the cells of the nodules showed so little differentiation, and appeared to possess such infiltrative power that the diagnosis was undoubtedly carcinoma.

The two points of great interest with regard to primary carcinoma of the liver which still require elucidation are its multicentric or unicentric origin and its relation to cirrhosis.

With regard to the first point the following evidence may aid in its solution. In an extremely cirrhotic liver three nodules were discovered which were proved histologically to consist of primary carcinoma of the hepatoma or liver cell type. Two of them (*a* and *b*) were situated in the left lobe; microscopic examination of the intervening tissue disclosed carcinomatous masses filling the vascular channels. It is therefore probable that either *a* metastasized from *b*, or that each appeared independently of the other. The occurrence of nodule *c* at a distant point in the right lobe sheds additional light on the problem. Both *b* and *c* were practically of the same size. Except for the areas immediately surrounding the capsules, complete microscopic examination of the cirrhotic tissue between *b* and *c* revealed no definite carcinomatous tissue or any tumor thrombi in the intervening vascular or lymphatic channels. The usual route by which these carcinomas spread is by either the portal or hepatic veins, but both these channels were quite free.

How then did the nodules *b* and *c* reach their respective positions? At this point it is important to bring forward an anatomic fact about the liver which has been demonstrated in another connection (McIndoe). From a vascular or biliary point of view the liver is a symmetrical organ with right and left lobes definitely supplied by the right and left branches of the portal vein and hepatic artery, and drained by the two branches of the bile duct. The line of demarcation runs through the middle of the gall bladder fossa to the point of entrance of the hepatic veins into the inferior vena cava. This has been proved to be constant for a large series of normal livers injected through the separate branches, and it bears no relationship to the usually described arrangement of five lobes (Figs. 9 and 10). Not only the afferent vascular channels but the bile ducts participate in this bilateral arrangement, so that the liver may be

said to consist essentially of two more or less equal halves with distinct vascular supply and biliary drainage. No vessels larger than capillaries cross the boundary line separating the two lobes except in the case of the hepatic artery, where there is a slight arteriolar anastomosis from side to side. It has also been shown that the lymphatics lie along the distribution of portal spaces and the hepatic veins, and that the flow is efferent in both cases (Helvestine, Lee).

The nodules *a* and *b* can be seen to lie well within the left half of the liver, while *c* lies in the right half. By microscopic examination, tumor thrombosis and embolism were found between *a* and *b*, but none between *b* and *c*. Direct extension may therefore be excluded. The chances of embolism from side to side are remote, for not only must the embolus have traversed the capillaries at the line of demarcation, but it must have done so against the vascular flow in all three channels including the lymphatics. The wide separation of the nodules almost precludes the chance of spread by way of the slight arteriolar anastomosis. The possibility of an embolus being swept into the inferior vena cava and through the general circulation until it finally lodged in the right lobe, or *vice versa*, is also very remote. As a general rule, with these tumors extension is direct within the liver and metastasis to distant organs uncommon even in the most advanced cases. The alternative probability is therefore that the nodules in the left lobe arose independently of those in the right, thus making them multicentric in origin.

Concerning the relationship of the cirrhosis to carcinoma little need be said. For Sabourin and the majority of subsequent writers, carcinoma follows in the footsteps of previous cirrhosis; for Hanot and Gilbert, cancer and cirrhosis develop simultaneously under the influence of the same irritating agent; and for Lancereaux and Wegelin, the cirrhotic lesions are secondary to the cancer. Here there can be but little doubt that the cirrhosis with its twelve-year history preceded the onset of carcinomatous change, a fact confirmed by the chronicity of the inflammatory reaction and the large amount of dense fibrous tissue found histologically. Moreover, all of the cirrhotic areas showed the most extreme hyperplastic changes, sometimes generalized and sometimes peripheral. Nuclear division has been so rapid that as many as nine nuclei were found in one hepatic cell. They were also more basophilic and hyperchromatic

than usual, tending to be larger and to exhibit one or more nucleoli with a distinctness and irregularity which are regarded by Broders as evidence of more marked hyperplasia and possibly precancerous change. Although, as has been frequently stated, the interpretation of multiple nodules of hyperplastic cells, as indicating multiple foci of carcinomatous change, should be made guardedly, yet the occurrence of this alteration in areas bearing such an anatomic relationship to each other makes the possibility somewhat stronger. It seems probable, therefore, that the carcinoma was superimposed on the extreme hyperplastic change resulting from severe generalized chronic hepatitis.

Summary and Conclusions

A case of long-standing atrophic cirrhosis of the liver has been described, in which early carcinomatous change of the hepatoma or liver cell type had taken place, and in which death was the result of the cirrhosis. The carcinoma was a secondary change. Two carcinomatous nodules were found in the right and left lobes of the liver, respectively, with no evidence of a common thrombotic or embolic origin. They were in areas of independent vascular supply and lymphatic drainage. In the absence of any possible channel permitting direct or embolic extension, these nodules are believed to have arisen independently of each other, and to represent a carcinoma of multicentric origin.

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DESCRIPTION OF PLATES

Plate 103

Fig. 1. Longitudinal section of liver showing marked atrophic portal cirrhosis with the nodules *a*, *b* and *c* distributed in the right and left lobes.

Fig. 2. Section of liver showing the remainder of nodule *c*.

Plate 104

Fig. 3. Bile capillaries in tumor distended with fluid and inspissated bile. × 250.

Fig. 4. Masses of tumor cells showing tubule formation. Note capillary network and absence of stroma. × 300.

Fig. 5. A multinucleated tumor cell. × 1000.

Fig. 6. A mitotic figure from nodule *b*. × 1000.

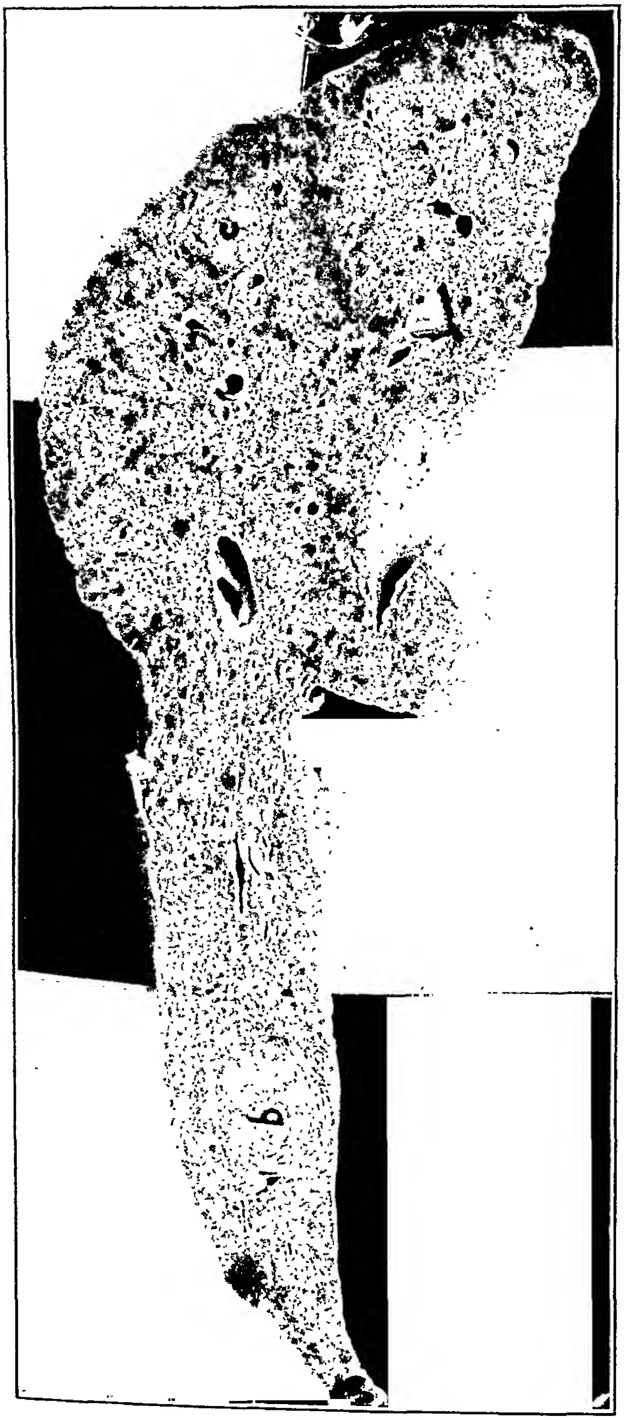
Fig. 7. A mitotic figure from nodule *c*. × 1000.

Fig. 8. Edge of nodule *c*, showing liver cells on right side and tumor cells on left. Direct invasion of parenchyma without the intervention of any capsule. × 250.

Plate 105

Fig. 9. Corrosion specimen of the two branches of the portal vein to show the independence of the two sides.

Fig. 10. Corrosion specimen of the two branches of the hepatic artery to show the definitely bilateral blood supply. Line of demarcation corresponds to the portal vein.



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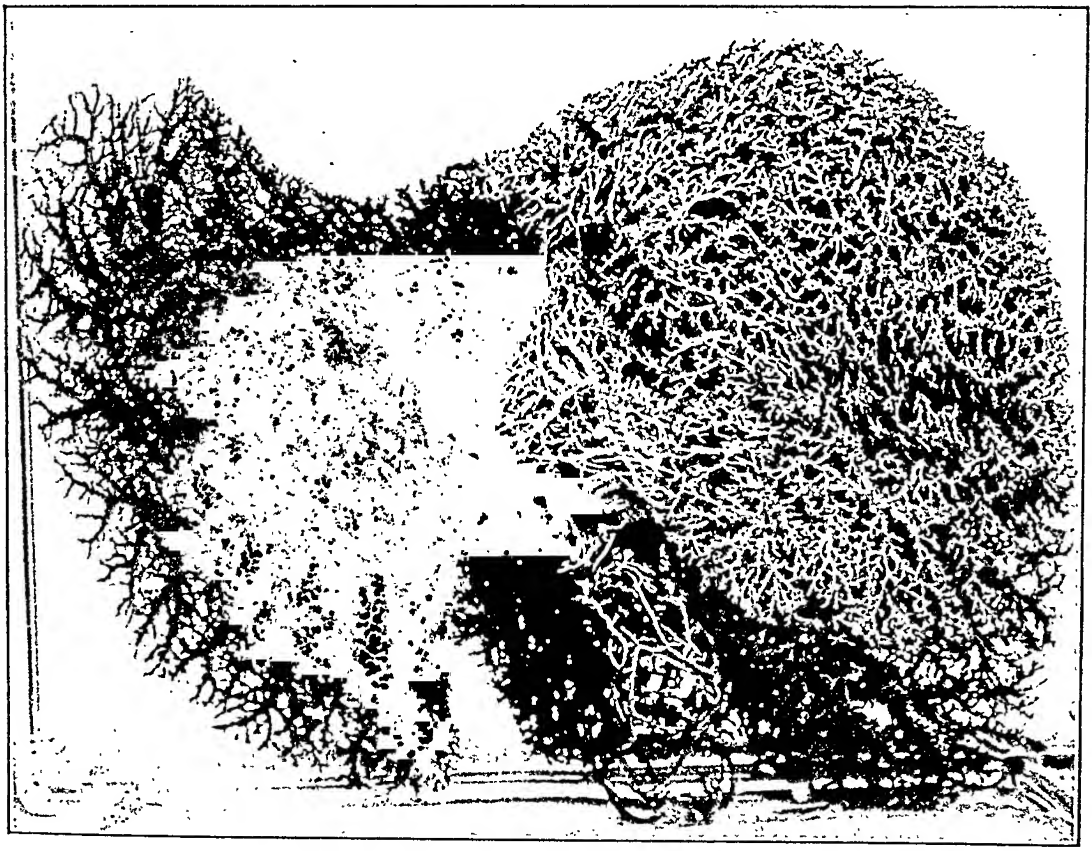
McIndoe and Counseller

Primary Carcinoma of the Liver



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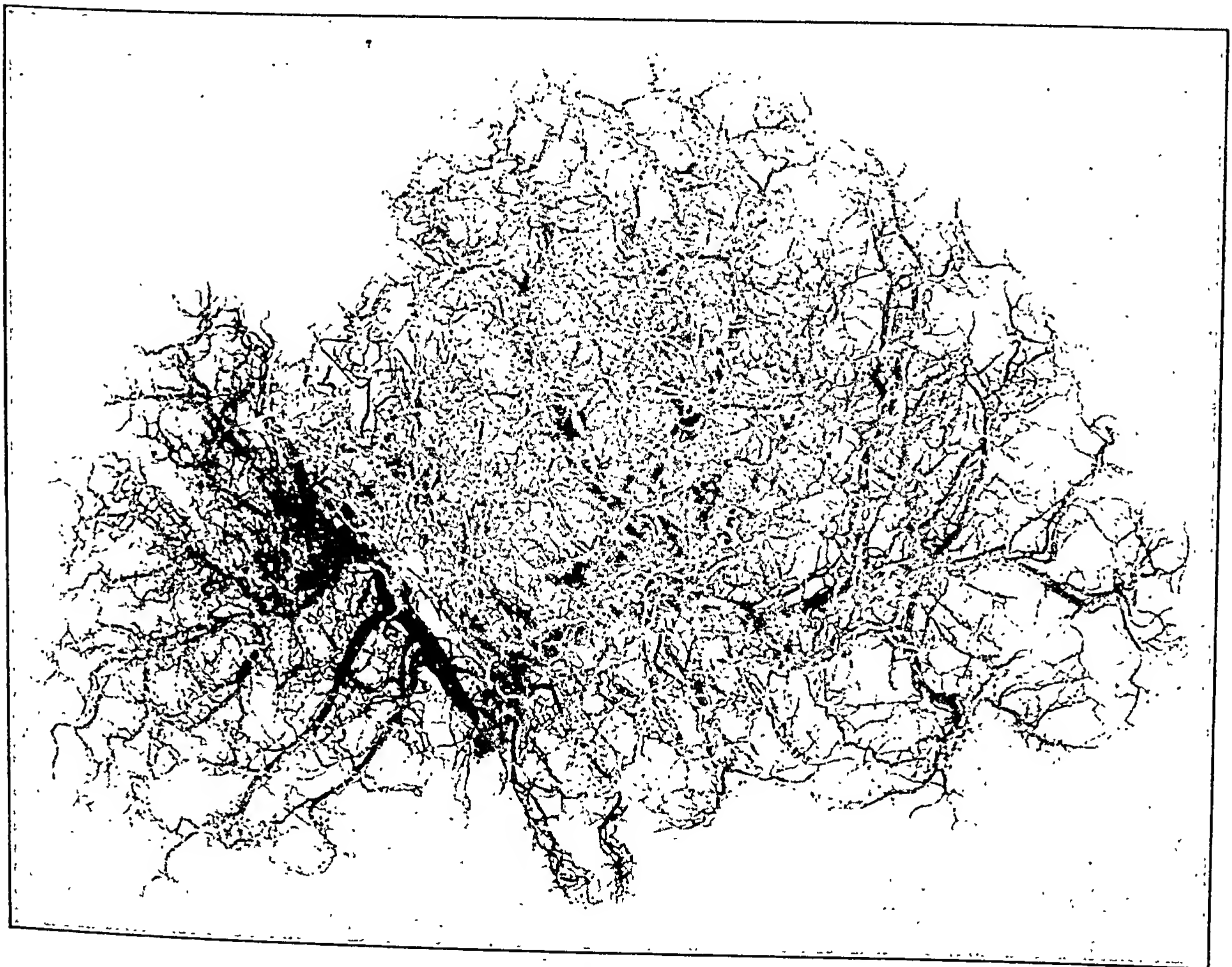

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McIndoe and Counseller

Primary Carcinoma of the Liver

STUDIES ON THE VASA VASORUM *

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Some observations have been made during the course of an investigation concerning the vasa vasorum, which seem to be of sufficient interest to report. Briefly stated, the experimental procedure consisted in the injection of the vessels of different animals with India ink gelatin. It was found possible to demonstrate the vasa vasorum by injecting the gelatin into the lumen of the vessel. More satisfactory preparations were obtained, however, by injecting the vasa directly through specially devised glass cannulae. Specimens were rendered transparent by Spalteholtz' method. The immediate presentation has to do with observations made on the aorta of the horse and the dog.

In the aorta of the horse, the vasa vasorum are found to penetrate through the media to the intima, where they terminate abruptly. Fig. 1 represents a transverse section from a portion of the aorta of the horse, with one of the larger vasa dipping down from the adventitia toward the intima. The section illustrates how the finer capillaries tend to spread out in a direction parallel to the circular muscle fibers and elastic tissue plaques, between which they run. Fig. 2 is an enlargement of the preceding picture and shows one of the branches spreading out into its finer arborizations. A longitudinal strip from the same aorta (Fig. 3) shows the abrupt line marking off the vascular media from the avascular intima. In Fig. 4 a diffuse network of vessels is seen in the adventitia of this aorta, with arterioles and venules running side by side.

In the aorta of the dog there are three outstanding considerations of interest. Vasa vasorum have been observed to arise directly from the lumen of the ascending aorta and supply this portion of the vessel. Fig. 5 shows one such vessel injected directly through the

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The data presented in this communication are taken from the dissertation presented to the Faculty of the Yale University School of Medicine in partial fulfillment of the requirements for the Degree of Doctor of Medicine.

small opening in the aorta at *a*, and also an extensive anastomosis including a branch *b* anastomosing with one of the coronary arteries.

That these openings occur normally in the aorta of the dog is indicated by the fact that the examination of the aortas of twenty-one animals revealed their presence in all but one. The aorta in this instance was observed to be the seat of extensive sclerotic change. The number of openings varied markedly, one aorta showing seven, while in several aortas only one opening was present. The commonest number was five, present in six of the aortas. Whether or not the absence of openings, mentioned in the one instance, is of significance in its association with the sclerotic change remains for further investigation to elucidate.

The commonest origin of the vasa vasorum of the aorta is from the branches of that vessel. Arising usually a few millimeters from the mouth of the branch, they run back to spread out on the surface of the larger vessel. This is shown in Fig. 6 which represents an aorta injected through its lumen, after each intercostal artery had been tied off, then opened and laid flat during the clearing process. The dark spots represent the intercostal vessels, the vasa vasorum running back from them to the aorta. The same arrangement is true with regard to the coronary arteries which give off vasa to the base of the aorta.

In addition to the above, a third point of interest was found in the highly vascularized pads of fat at the base of the dog's heart. The pads frequently assume the most bizarre shapes and appear to fill in every crevice between the auricles and ventricles and the surrounding pericardium. Frequently one of the discrete openings of the vasa is found in the aorta immediately underneath one of the fat pads. When injected, the pads fairly balloon out, because of the filling of the vascular net which permeates them. See Figs. 7, 8 and 9.

Summary

1. Illustrations are given showing the vasa vasorum in the aorta of the horse extending through the media to the intima.

2. Vasa vasorum are pictured arising from the intercostal arteries of the aorta of the dog.

3. An illustration showing a vas vasis arising directly from a discrete opening in the ascending portion of the aorta of the dog is given.

4. Fat pads at the base of the aorta of the dog are described and illustrated.

5. Data are given regarding the occurrence of discrete openings of the vasa vasorum in the aorta of the normal dog and the question is raised whether the absence of these openings is significantly associated with sclerotic change in the aorta.

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Plate 106

Fig. 1. Transverse section, horse's aorta. × 4.
Fig. 2. Transverse section, horse's aorta. × 10.
Fig. 3. Longitudinal strip, horse's aorta. × 3.

Plate 107

Fig. 4. Adventitial vessels, horse's aorta. × 10.
Fig. 5. Discrete opening of vasa vasorum in ascending portion of dog's aorta. × 2.
Fig. 6. Vasa vasorum arising from intercostal arteries, dog's aorta. × 2.

Plate 108

Fig. 7. Transverse section from ascending portion of dog's aorta, showing fat pads. × $3\frac{1}{2}$.
Fig. 8. Fat pad on ascending portion of dog's aorta; pulmonary artery at *P*. × $3\frac{1}{2}$.
Fig. 9. Enlargement of tip of fat pad; epicardial covering at *e*. × 12.

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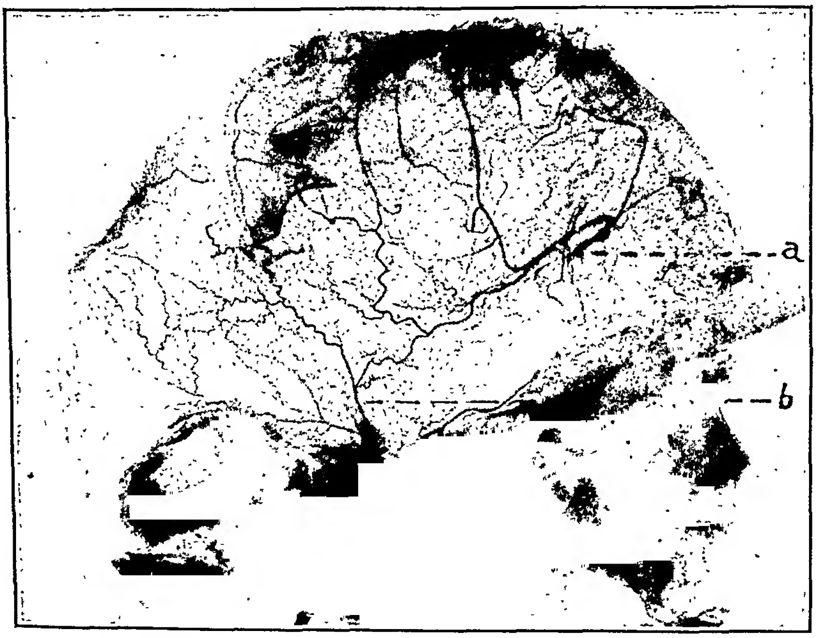

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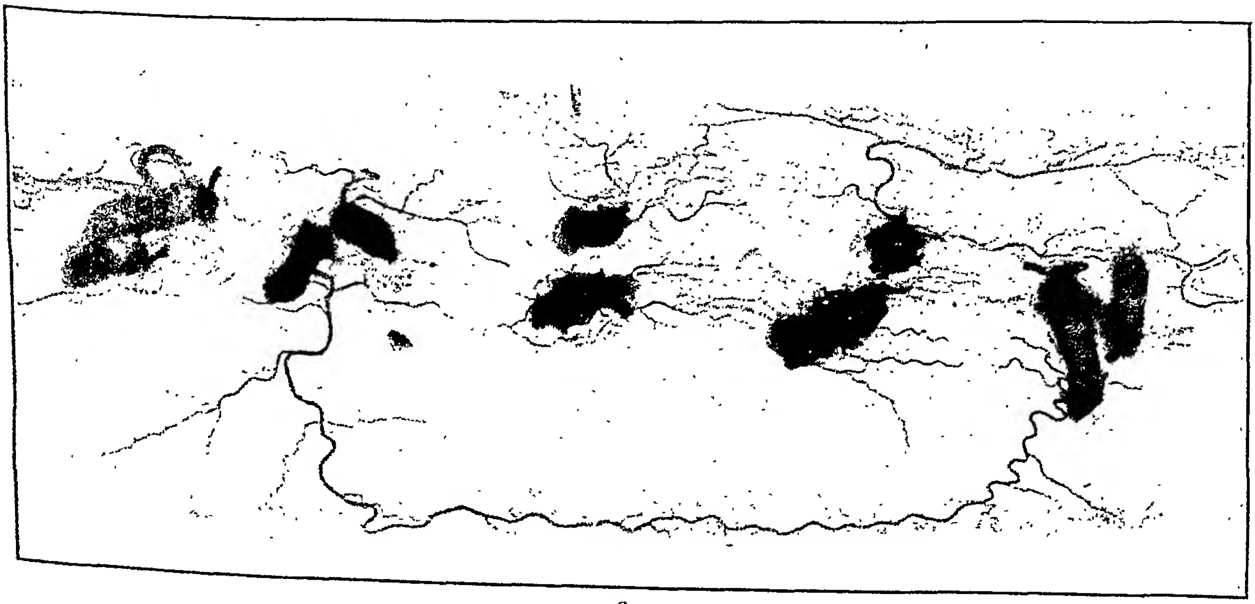

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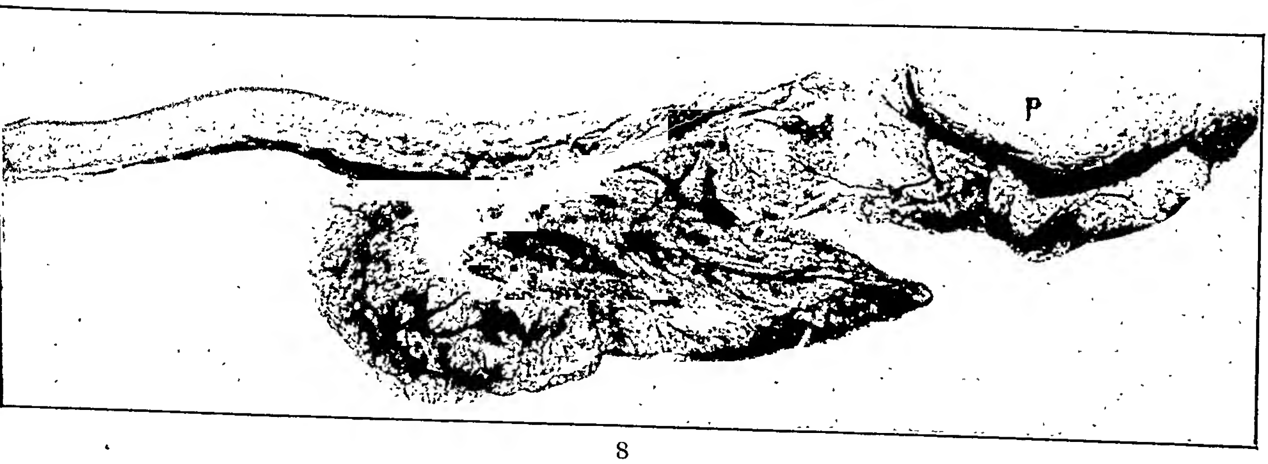

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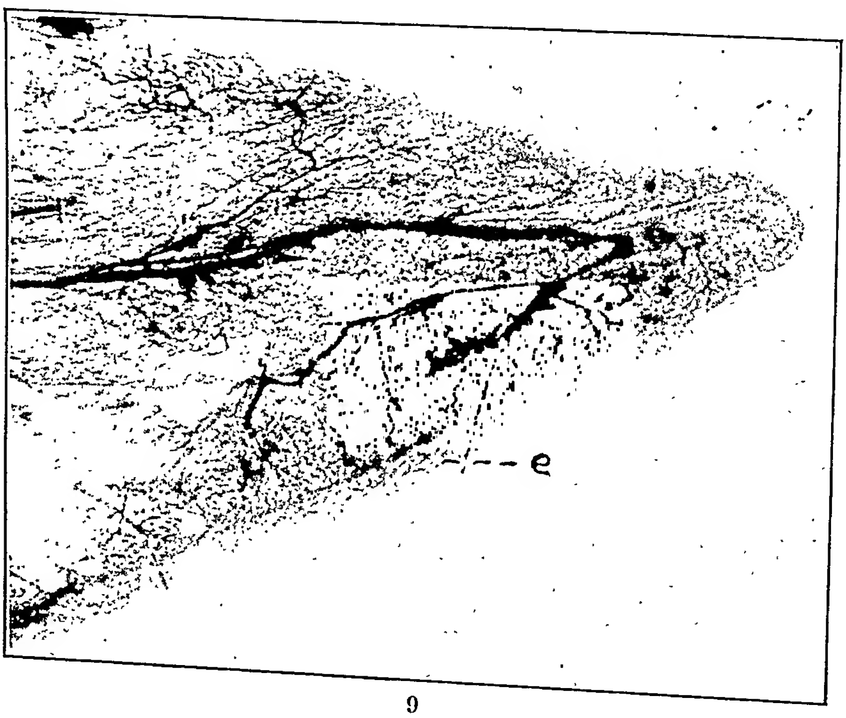

Woodruff

9

THE SIGNIFICANCE OF GIANT CELLS IN THE INTRADERMAL TUBERCULIN REACTION *

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National Research Council Fellow in Medicine
and
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Numerous writers in studying the intradermal tuberculin reaction in tuberculous guinea-pigs have called attention to the curious phenomenon usually designated as the "lighting-up of a previous site of reaction." From time to time in retesting a tuberculous animal either intracutaneously or by other routes, an intradermal tuberculin reaction obtained several days previously may be observed to show an increase in surrounding erythema. No very satisfactory explanation of this interesting phenomenon has as yet been offered. Coca [1] presents a theory which may be briefly summarized as follows: The character of the histologic changes that take place at the site of the local tuberculin reaction is not without significance with respect to its anaphylactic nature. It appears that in some of these lesions that were subjected to microscopic study, the changes resembled those of tuberculosis while in others they could not be distinguished from the typical lesions of that disease. The pathology of the local tuberculin reaction appears, thus, to be that of tuberculosis, and the specific character of this lesion may have some relation to the instances of a "lighting-up" at the sites of previous injections after a subsequent one. Such a change is conceivably due to a mechanism similar to that which underlies the "focal reaction."

In support of his contention Coca quotes the observations of Bandler and Kreibich,[2] and Daels.[3] Reference to this first paper, however, reveals the fact that these authors did not regard their histologic findings as indicative of a local tubercle formation. They state quite clearly that the giant cells and epithelioid cells seen by them differ from the Langhans type of cell and they note especially their prevalence in fat-containing tissues. Daels, however, did consider the reaction to be of the nature of a local tubercle and believed it due to the presence of occasional killed bacilli and fragments of

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bacilli in the tuberculin. An attempted differentiation between the Langhans cell and the foreign body giant cell may be wholly superfluous since Medlar[4] sees in the giant cell of tuberculosis nothing more than a foreign body giant cell. He states that giant cells are an indication of a reparative process in small areas of caseation or of simple necrosis of tissue — a reaction to a foreign body. This contention would appear to vary considerably from the concepts recently enunciated by Cunningham, Sabin and their co-workers,[5] these latter believing the tubercle to be a highly specific structure, indicative of a peculiar symbiosis between the tubercle bacillus and the monocyte.

The authors, in an attempt to study the question of tuberculin desensitization in guinea-pigs had occasion to examine histologically a number of intradermal tuberculin reactions. The usual giant cells were observed and it became desirable to analyze the factors responsible for their formation. Ten per cent old tuberculin was used to elicit the reaction and injections were made intracutaneously, enough tuberculin being used to produce a bleb 6 or 7 mm. in diameter. Tissues were available for study at hourly stages from the ninth to the twenty-third hour and at 1, 2, 3, 4 and 6 days.

The early stages of the reaction may be passed over briefly. They are characterized by marked edema, fibrin deposit, exudate of polymorphonuclear leucocytes and eosinophiles and necrosis of certain tissues. The necrosis is dependent upon the intensity and depth of the reaction. Necrosis of epithelium and fat cells occurs with great regularity. In intense reactions one sees necrosis of collagen; in deeper reactions some necrosis of muscle with invasion by polymorphonuclear leucocytes occurs. The subsequent course of events is best described by reference to the accompanying photomicrographs. Fig. 1, taken from an early skin test, shows a group of fat cells; about two of these cells is a subsiding reaction; immediately outside the clear area of fat is a narrow zone of polymorphonuclear leucocytes, the cells which surrounded the fat during the stage of acute reaction. About these cells is a second zone consisting of endothelial leucocytes attracted by necrotic fat. Fig. 2 shows a slightly later stage (four days); here essentially the same condition is found but the endothelial leucocytes surrounding fat and polymorphonuclear leucocytes have fused to form small foreign body giant cells; in addition to these small giant cells there are two large

giant cells, one of which contains fat droplets and partially surrounds a fat cell. Numerous fibroblasts are present. Gradually polymorphonuclear leucocytes disappear and the resultant picture is that of giant cells surrounding fat or containing globules of fat (Figs. 3 and 4). Still later all traces of fat may vanish leaving giant cells embedded in dense fibrous stroma (Fig. 5).

Foreign body giant cells may form about other structures than fat. We have observed them to a very limited extent about old fibrin and necrotic collagen. Necrosis of fat cells, however, is the chief exciting agent in their formation.

If such an etiology is correct, then non-specific necrosing agents may be expected to produce a similar result and this is in fact the case. Mallein in full strength (250 mg. per cc.) produces in the skin of normal guinea-pigs a reaction grossly similar to the intracutaneous tuberculin reaction and in its later stages the microscopic picture in every way resembles that of the tuberculin reaction. Giant cells are formed in identical fashion. Consequently, there is nothing specific in the formation of these giant cells *per se.* That giant cells may form following necrosis of tissue in actual tuberculous lesions, is an undoubted fact, but there too Medlar has justly doubted any specific character. We likewise would regard them as non-specific structures developing in response to necrosis, and see no reason for drawing immunologic conclusions from their presence.

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Plate 109

Fig. 1. Early intradermal tuberculin reaction; a subsiding acute inflammatory reaction about fat cells. $\times$ 500.

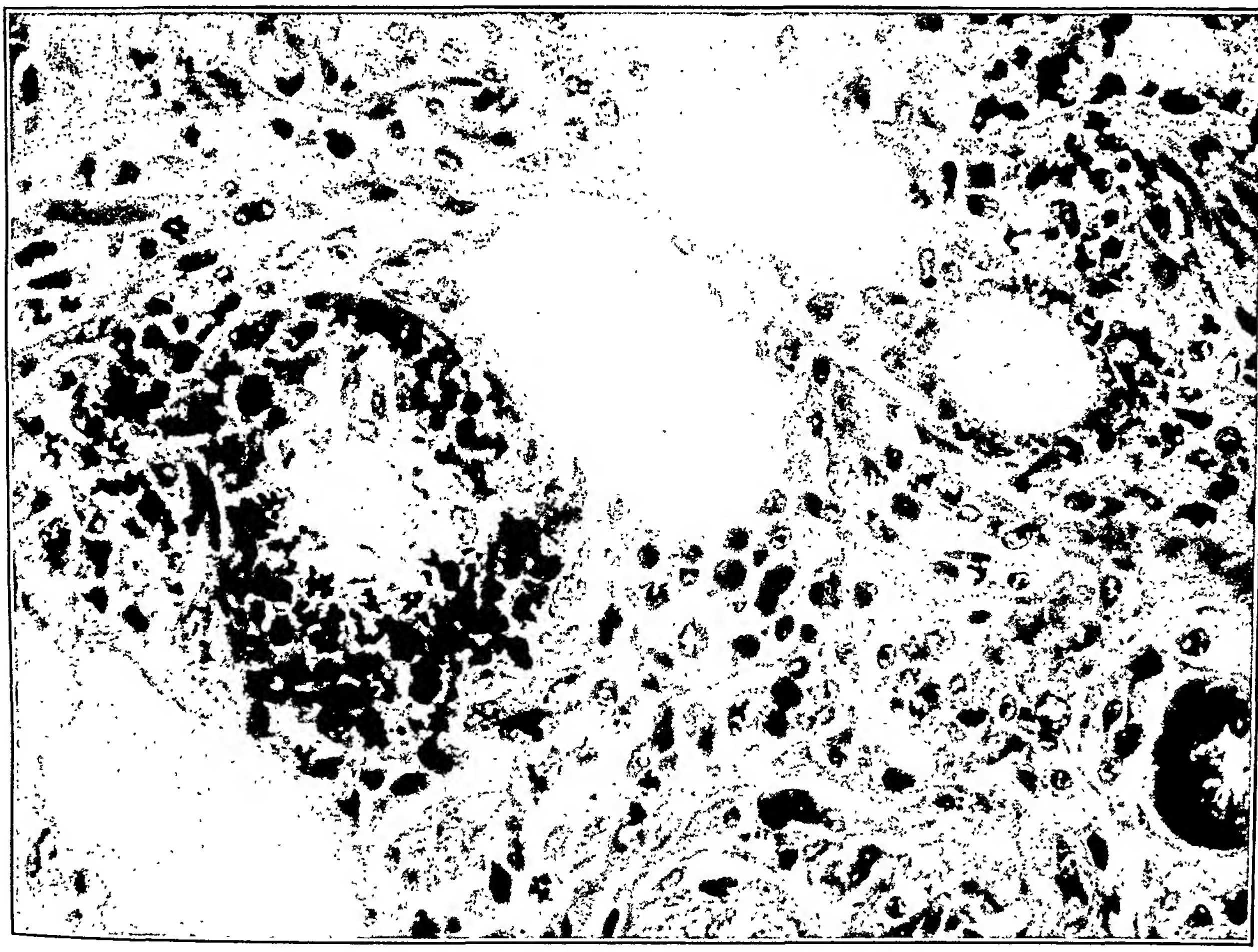
Fig. 2. Further subsidence of reaction, four day skin test. Giant cell formation about fat. $\times$ 500.

Plate 110

Fig. 3. Large giant cells about fat; same animal as Fig. 2. $\times$ 500.

Fig. 4. Numerous fat globules in giant cells, four day skin test. $\times$ 500.

Fig. 5. Older giant cells remaining in dense scar tissue on the sixth day. $\times$ 250.



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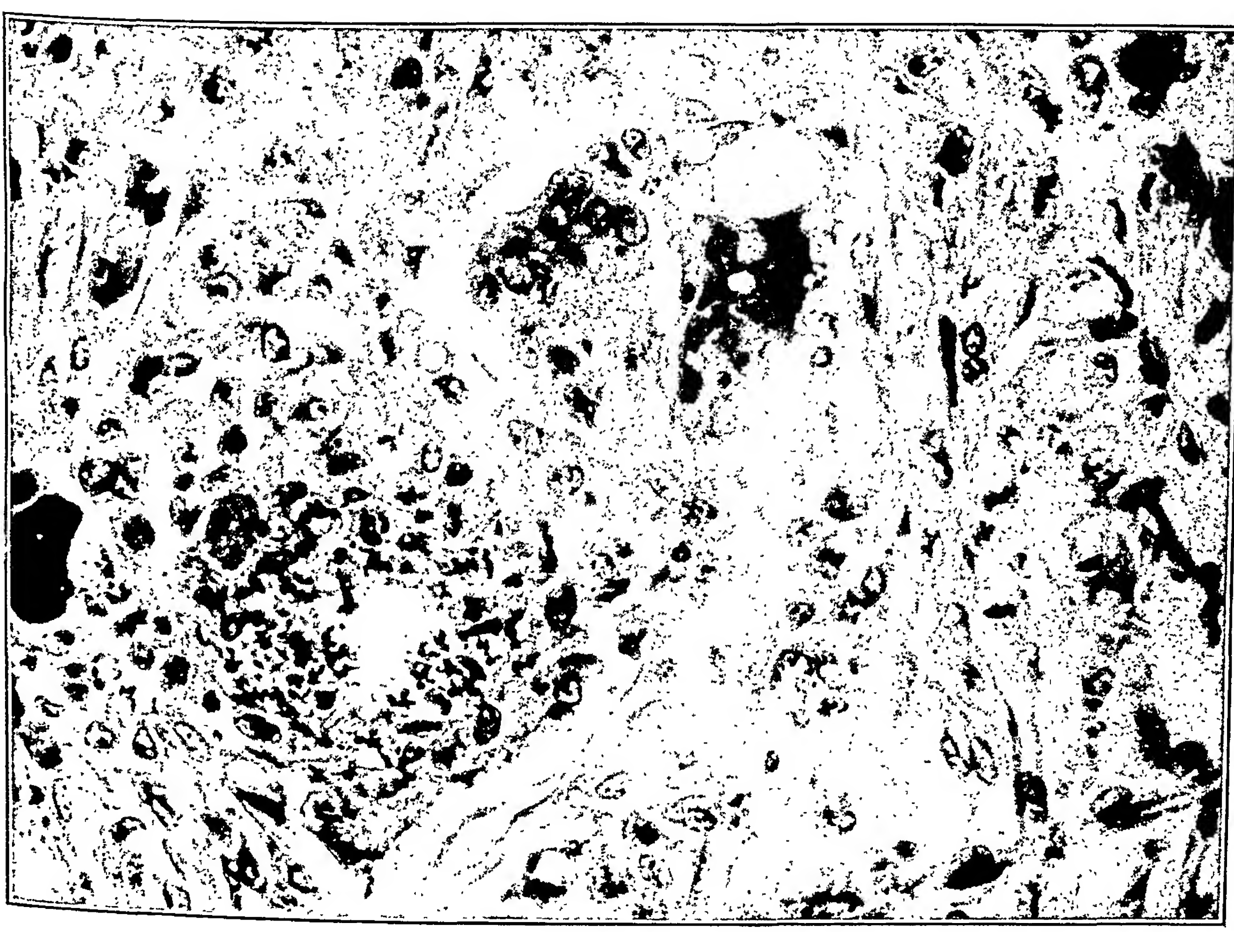

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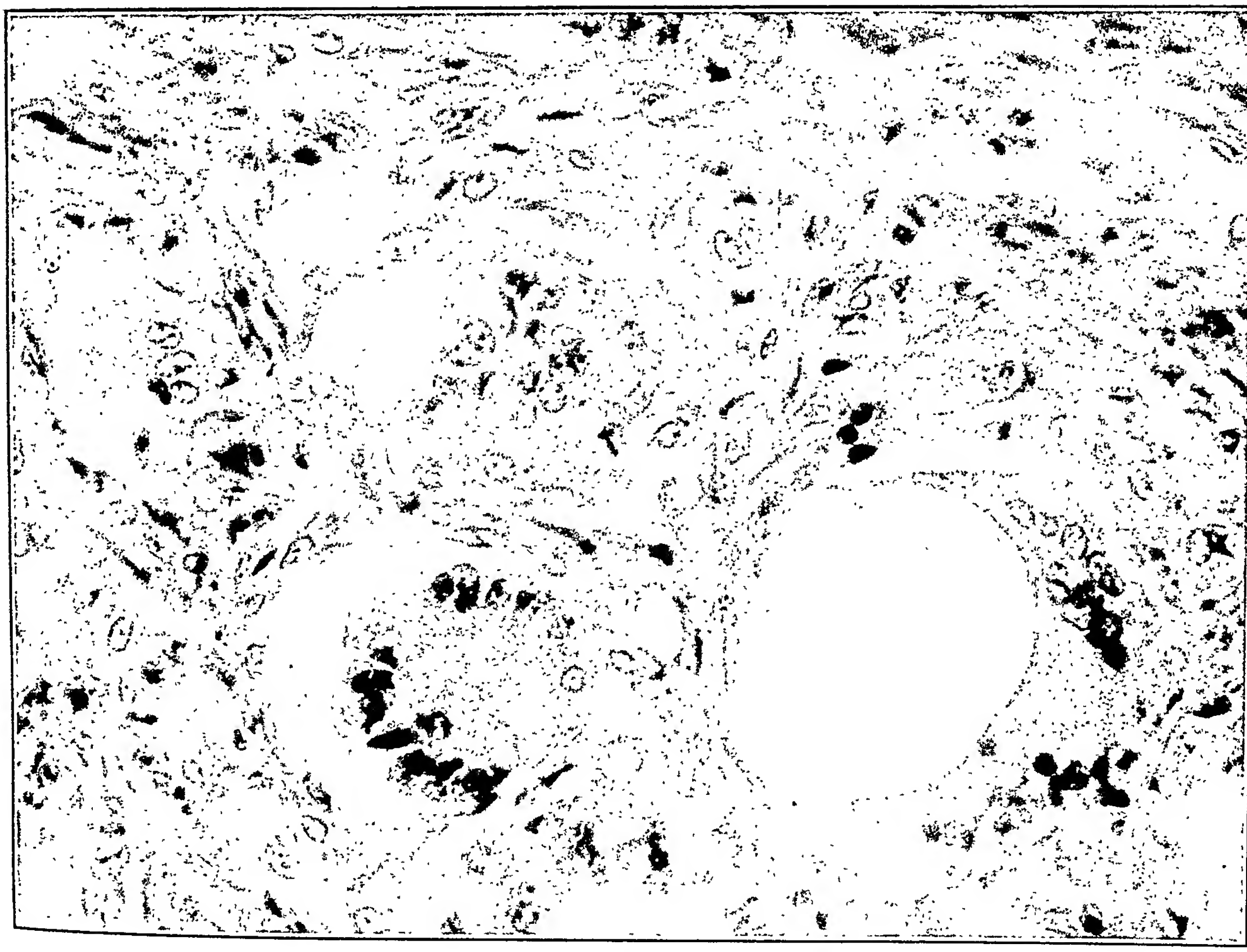
Stewart and Rhoads

Giant Cells in the Tuberculin Reaction



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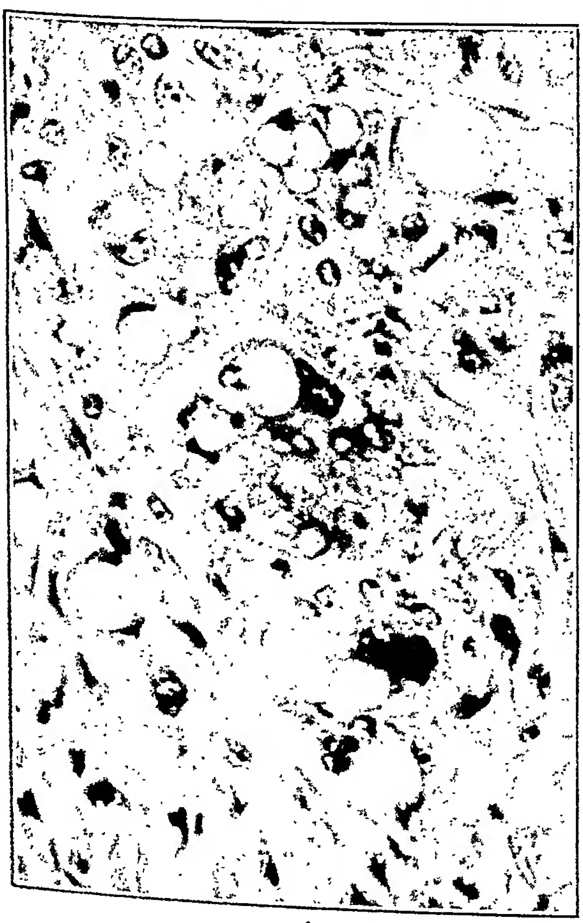

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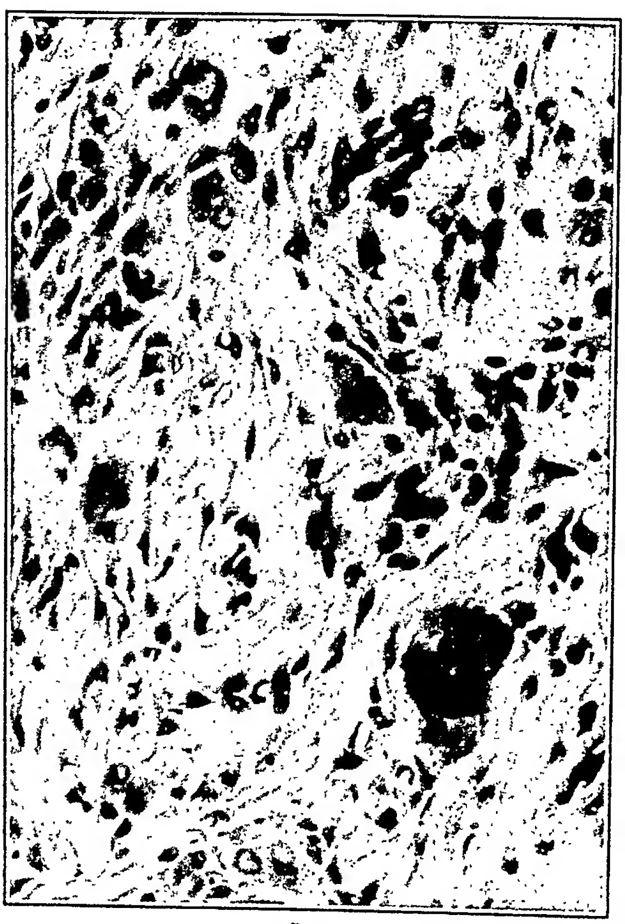

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Stewart and Rhoads

Giant Cells in the Tuberculin Reaction

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* Abstract of paper presented at the meeting of the Association of Pathologists and Bacteriologists held at Albany, N. Y., April 2 and 3, 1926.

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